THE INVOLVEMENT OF AMYGDALA NEURONS AND AMYGDALOID DOPAMINERGIC AND GLUTAMATERGIC RECEPTORS IN THE ACQUISITION AND REINSTATEMENT OF FEAR-POTENTIATED STARTLE IN RATS

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Figure 1 above depicts how auditory information reaches the amygdala. Note that auditory input is transmitted to the amygdala either via the sub-cortical and direct thalamo-amygdaloid route (left portion of Figure 1.) or via the longer and indirect cortical route that involves the thalamo-cortico-amygdaloid auditory pathways (right portion of Figure 1). The direct route involves the mdMGN (medial division of the medial geniculate nucleus), SGN (suprageniculate nucleus) and the PIN (posterior intralaminar nucleus) sending auditory information to the lateral amygdaloid nucleus (LA), amygdalostriatal transition area (AStr), and the basolateral amygdala (BLA). However, some auditory information is also sent to Te2 and Te3 before being transmitted to the perirhinal cortex (PRh) and on to the amygdala. The indirect cortical route begins in the ventral subdivision of the medial geniculate nucleus of the thalamus (vdMGN). From the vdMGN, auditory information is first transferred to Te1 and then on to Te2 and Te3 of the temporal cortex. Auditory signals are then sent to the anterior PRh before finally being transmitted to the AStr, LA, BLA and basomedial amygdaloid nucleus (BMA).

Figure 2 above provides a schematic description of how visual information reaches the amygdala. Visual sensory input reaches the amygdala through either direct thalamo-amygdaloid pathways (left portion of Figure 2) or via indirect thalamo-cortico-amygdaloid cascades (middle to right portion of Figure 2). The lateral posterior nucleus of the thalamus (LPN) sends visual information directly to the LA and BLA, but some visual information is transmitted to the Te2 before reaching some amygdaloid regions (e.g. AStr, LA, and BLA). The thalamo-cortico-amygdaloid visual cascade begins with the lateral geniculate nucleus of the thalamus (LGN) transmitting visio-sensory information to Oc1 (primary occipital cortex), Zona Incerta (ZI) and the suprageniculate nucleus of the thalamus (SGN). Oc1 sends the visual information to the secondary occipital cortex (Oc2). Oc2 in turn passes the visual input on to Te2. Te2 then transmits the visual information either directly to several amygdaloid nuclear regions (i.e. AStr, LA, BLA) or to the anterior and posterior portions of the perirhinal cortex (PRh). Visual information from the SGN also eventually reaches the PRh via the Reuniens Nucleus (RE). The visual information in the PRh is then sent to various amygdaloid nuclei (e.g. AStr, LA, BLA, BMA, CeA) to complete the visual cascade. Note CeA = central nucleus of the amygdala, LA = lateral nucleus of the amygdala, AStr = amygdalostriatal transition area, BLA = basolateral amygdala, BMA = basomedial amygdaloid nucleus.

Figure 3 above provides a schematic depiction of how somatosensory information reaches the amygdala. Somatosensory information reaches the amygdala three or event four ways. The first is directly through the mdMGN (medial division of the medial geniculate nucleus of the thalamus), the SGN (suprageniculate nucleus of the thalamus), and the PIN (posterior intralaminar nucleus of the thalamus). mdMGN, SGN, and PIN together send somatosensory input to the AStr, LA, BLA, and CeA. These thalamic nuclei also pass somatosensory information on to Te2. The second somatosensory cascade is indirect (i.e. via thalamo-cortico-amygdaloid pathways) whereby the ventral posterolateral nucleus of

the thalamus (VPL) and the ventral posteromedial nucleus of the thalamus (VMP) send sensory information to the primary somatosensory area (S1/Par1). S1/Par1 sends information to the secondary somatosensory area S2/Par2. S2/Par2 in turn sends somatosensory information to Te3. Some of the information in Te3 is passed on directly to the amygdala (i.e. AStr, and LA), whilst some is sent to the posterior insula cortex. The third somatosensory cascade originates from the medial posterior complex of the thalamus (POM) and sends information directly to the BMA, central nucleus of the amygdala (CeA), and medial nucleus of the amygdala (MeA). The final way somatosensory information reaches the amygdala (i.e. BMA, CeA, and MeA) is directly via the pontine parabrachial nucleus (PBN)

Figure 4. The above figure portrays a schematic representation of how visceral and gustatory information reaches the amygdaloid complex. Basically, the visceral and gustatory information cascade to the amygdala begins with the solitary nucleus of the medulla transmitting visceral and gustatory information to the dorsal parvicellular subdivision of the ventral posterior nucleus of the thalamus (dVPN) and the ventral parvicellular subdivision of the ventral posterior nucleus of the thalamus (vVPN). Visceral and Gustatory information from the dVPN and vVPN is then sent to the anterior granular, dysgranular and agranular insular cortex. From these regions of the insula cortex, much of the visceral and gustatory information is sent to several amygdaloid nuclear groups. These include the LA, BLA, BMA and CeA. The insula cortex also sends visceral and gustatory information to the anterior cortical nucleus of the amygdala (CoA) and the medial nucleus of the amygdala (MeA).

Figure 5 (above) displays the three general ways nociceptive information reaches the amygdala complex. The first (depicted on the left) is the direct spino-thalamo-amygdaloid pain pathway. This pathway involves pain signals in the spinal cord being transmitted to several thalamic nuclei. These include the medial division of the medial geniculate nucleus (mdMGN), the posterior thalamic nucleus (PTN) and the posterior intralaminar nucleus (PIN). The mdMGN passes on pain information to the AStr, LA, and the lateral capsular subdivision of the central amygdaloid nucleus (CLC). Similarly PTN and PIN transmit nociceptive information directly to the LA and BLA. The second direct pain pathway to the amygdala involves the parabrachial nucleus (PBN) that sends information to the BLA, CeA, CLC and AStr (see right side of figure). The third nociceptive pathway is the spino-thalamo-cortico-amygdaloid system depicted in the middle of Figure 5. PIN, PTN, and VPN (ventral posterior nucleus of thalamus) receive input from the spinal cord and then pass this on to the primary somatosensory area S1. S1 sends pain information to the secondary somatosensory region (S2). Through a series of cascades S2 sends nociceptive information on to the parietal ventral area, the parietal rhinal cortex, the posterior granular and dysgranular insula, and the perirhinal cortex (PRh). Finally, several amygdaloid nuclei (i.e. LA, BLA, CLC and CeA) receive nociceptive information from the PRh and parietal regions.

Figure 6. The figure presented above provides a simple depiction of ascending amygdaloidal projections to some cortical and limbic brain regions. On the left of the figure the BLA can be seen projecting to the lateral and dorsal subdivisions of the bed nucleus of the stria terminalis [BNST(Ld)], the striatum, the posterior and medial nucleus accumbens [Nuc Acc (P&M)], the perirhinal cortex (PRh), the entorhinal cortex and the medial prefrontal cortex. The central nucleus of the amygdala (CeA) innervates the BNST(Ld), the substantia innominata (S.I.), the Septum and the Nuc Acc (middle of figure). Further to the right, the basomedial amygdala (BMA) is shown projecting to the medial and lateral divisions of the BNST and the anterior Nuc Acc, whereas the amygdalohippocampal area (AHA) innervates the ventral and lateral regions of the Septum. Finally, on the extreme right of Figure 6, the anterior cortical amygdaloid nucleus (CoA) can be seen projecting to the medial and lateral BNST (md-BNST), whilst the medial cortical amygdaloid nucleus (CoM) targets the md-BNST and the Septum.

Figure 7 presented above, depicts some of the key descending amygdaloid projections to the sub-cortical and midbrain structures. On the left of the figure the central nucleus and medial nuclei of the amygdala (CeA and MeA) project to the dorsal and midline thalamic nuclei and the centromedial nucleus of the thalamus. In the middle of the Figure 7, CeA and BLA send projections to the lateral hypothalamus (L.H.). Most important are the projections (depicted by 3 arrows) innervating the caudal L.H. close to the sub-thalamic nucleus. This pathway represents the caudal ventral amygdalofugal pathway to the midbrain and pons. This direct pathway to the reticularis pontis caudalis (RPC) is important for the FPS expression. Basically, efferents from the medial CeA and parts of the BLA travel to the substantia nigra (SN), the ventral tegmental area (VTA) and the ventrolateral periaqueductal gray (vlPAG). Some synaptic contacts are made in these midbrain areas. However, a majority of CeA and BLA efferents reach the RPC which is an important nucleus for mediating FPS in rats. Within the pons CeA and BLA efferents target the locus coeruleus (LC), the parabrachial nucleus (PBN) and the Raphe nucleus. Efferents from the CeA and BLA reach the nucleus of the solitary tract (N.Sol, Tr.) and the dorsal motor nucleus of the vagus (dMN vagus) and then enter the spinal cord. Further to the right, the medial nucleus of the amygdala (MeA) and the BMA innervate the ventromedial and dorsomedial hypothalamic nuclei (vm.H & dm.H). Finally, the anterior amygdaloid area (AAA) and the anterior cortical nucleus of the amygdala (CoA) innervate the shell and core of the ventromedial hypothalamic nuclei, whilst the CoA and the medial cortical nucleus of the amygdala (CoM) target the supraoptic, paraventricular, and parvicellular aspects of the paraventricular nucleus of the hypothalamus (PVN soop).

Figure 8. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the saline control group (N=12) of the fear-potentiated acquisition studies of Experiment 1A. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventral beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56

mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 9. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the 2.0 μg raclopride group (N=12) of the fear-potentiated acquisition studies of Experiment 1A. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventral beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 10. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the 4.0 μg raclopride group (N=12) of the fear-potentiated acquisition studies of Experiment 1A. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventral beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 11. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the 8.0 μg raclopride group (N=12) of the fear-potentiated acquisition studies of Experiment 1A. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventral beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 12. Mean white-noise intensity level in decibels that was used to induce acoustic startle responses in the saline control group (N=12) and the three raclopride drug groups (2.0 μg, 4.0 μg, and 8.0 μg; N=12 each). Presented above are the mean decibel values along with the S.E.M. ± for each drug treatment group.

Figure 13. Mean (S.E.M. ±) acoustic startle amplitudes for the saline group (N=12) and the three raclopride groups (2.0 μg, 4.0 μg, and 8.0 μg; N=12 each). Baseline startle amplitudes were obtained from the seventh baselining block which occurred 48 hours prior to either drug infusion or fear conditioning and testing.

Figure 14. Mean (S.E.M. ±) acoustic startle amplitudes recorded on each 4 noise-alone and 4 CS + noise trial test block as a function of infusion of saline (N=12) or raclopride (2.0 μg, 4.0 μg, and 8.0 μg; N=12 each) into the basolateral amygdala and fear conditioning. A fear conditioning block (8 light + footshock pairings) preceded each of the five test blocks allowing for a total of 40 fear conditioning trials to be administered across the five train-test blocks (★ P< 0.05 CS + noise relative to noise alone trials).

Figure 14(a). Mean (S.E.M. ±) acoustic startle amplitudes recorded on each 4 CS + noise trial type test block as a function of infusion of saline (N=12) or raclopride (2.0 μg, 4.0 μg, and 8.0 μg; N=12 each) into the basolateral amygdala and fear conditioning. As can be seen with this between-group comparison on just the CS + noise stimulus type, the saline rats exhibited higher startle responding on the CS + noise trials than did raclopride-infused rats. Double star symbols (★★) placed over each raclopride group's bar graph represents a statistically significant difference on CS + noise trials on each individual test block (★★ P<0.05 relative to the saline group's CS + noise scores on each test block). Clearly, the mean startle scores on the CS + noise trial type for the raclopride-infused rats were significantly smaller than that obtained from the saline-treated rats across most of the test blocks. This further illustrates that pretraining intra-BLA raclopride infusions prevented CS-induced startle from developing.

Figure 15. The grand mean (S.E.M. ±) of the difference scores ([CS + noise]-[noise-alone]) collapsed over the five fear acquisition test blocks after intra-basolateral amygdaloid infusion of either saline (N=12) or raclopride (2.0 μg, 4.0 μg, and 8.0 μg; N=12 each; ★ P< 0.05 relative to saline control group).

Figure 16. Mean (S.E.M. ±) acoustic startle amplitudes and difference score ([10 CS + noise]-[10 Noise-alone]) results for saline control (N=12) and raclopride (2.0 μg, 4.0 μg, and 8.0 μg) groups (N=12 each) on the retention test conducted 48 hours after bilateral basolateral amygdaloid infusions and 5 fear conditioning/test blocks (★ P<0.05 CS + Noise relative to the Noise-alone trial type; ✦ P<0.05 difference scores of raclopride drug groups 2.0, 4.0 and 8.0 μg relative to the saline group's difference score). The double star symbols (★★) placed over the raclopride 4.0 μg and 8.0 μg groups denote a statistically significant between-group difference on the CS + noise trial type relative to the saline group (★★ P<0.05 relative to the saline group's CS + noise mean startle score on the retention test).

Figure 17. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) of the saline and raclopride (2.0 μg, 4.0 μg, and 8.0 μg) groups (N=12 each) of the retention test administered 48 hours after basolateral amygdaloid drug infusion and fear conditioning/testing (★ P<0.05 relative to the saline control group).

Figure 18. The mean movement amplitude (S.E.M. ±) recorded during the presentation of 5 blocks of 8 light + footshock fear conditioning trials to rats that were infused with either saline or one of three doses of raclopride (2.0 μg, 4.0 μg or 8.0 μg) into the basolateral amygdala.

Figure 19. Mean (S.E.M. ±) acoustic startle amplitude of the last 4 noise-alone trials prior to fear conditioning (Preshock Noise) for the raclopride (2.0 μg, 4.0 μg, and 8.0 μg) and saline basolateral amygdala infusion groups versus the average startle amplitudes obtained on each of the 5 test blocks containing 4 noise-alone trials presented after light + footshock pairings (depicted as Noise 1 to Noise 5). The symbol (★ ; $P<0.05$) represents significant differences of the post-shock acoustic startle means (Noise 1 to 5) relative to the preshock startle means for each drug treatment condition.

Figure 20. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the saline control group (N=9) of Experiment 1B that examined raclopride's effects on acoustic startle responding. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 21. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 8.0 μg group (N=9) of Experiment 1B that examined raclopride's effects on acoustic startle responding. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A. P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 22. Mean (S.E.M. ±) acoustic startle amplitudes on the pre-infusion and the post-infusion acoustic startle tests for animals infused with either saline or an 8.0 μg dose of raclopride into the basolateral amygdala (★ $P<0.05$ relative to predrug infusion startle).

Figure 23. Mean movement amplitudes (S.E.M. ±) recorded 100 milliseconds before light-alone onset (prelight movement in total darkness) and 100 milliseconds after the onset of the 3.5 second light presentation (post light movement) that illuminated the test chamber. The pre-movement and post-movement means were calculated from 40 light-alone presentations (no footshock).

Figure 24. Mean startle amplitudes (S.E.M. ±) for 10 light + noise trials and 10 noise-alone trials that were used to assess the effects of light only (no footshock) presentations made 48 hours earlier on fear-potentiated startle. The mean difference scores (S.E.M. ±) ([10 light + noise]-[10 noise-alone]) demonstrate that 40 light only presentations do not

produce fear-potentiated startle when they are not repeatedly paired with aversive footshock.

Figure 25. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the saline group (N=13) for the fear-potentiated startle acquisition control Experiment 1D. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 26. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 4.0 μg group (N=13) for the fear-potentiated startle acquisition control Experiment 1D. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 27. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the SCH 23390 R+ 2.0 μg group (N=13) for the fear-potentiated startle acquisition control Experiment 1D. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom: -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 28. Mean (S.E.M. ±) baseline startle amplitudes recorded on the seventh baselining block for the saline, raclopride 4.0 μg, and SCH 23390 2.0 μg experimental groups (N=13 per experimental group).

Figure 29. Mean (S.E.M. ±) acoustic startle amplitudes recorded on each 4 noise-alone and 4 CS + noise trial test block as a function of infusion of saline, raclopride (4.0 μg) or SCH 23390 (2.0 μg) into the basolateral amygdala and fear conditioning. A fear conditioning block (8 light + footshock pairings) preceded each of the five test blocks allowing for a total of 40 fear conditioning trials to be presented across the five train-test blocks (★ $P<0.05$ CS + noise relative to noise-alone trials).

Figure 29(a). Mean (S.E.M. ±) acoustic startle amplitudes recorded on each 4 CS + noise trial type test block as a function of infusion of saline, raclopride (4.0 μg) or SCH 23390 (2.0 μg) into the basolateral amygdala and fear conditioning. As can be seen with this between-group comparison on just the CS + noise stimulus type, the saline-infused rats exhibited higher startle on the CS + noise stimulus type trials on most of the test blocks than did rats infused with either raclopride or SCH 23390. Double star symbols (★★) placed over the raclopride and SCH 23390 group's bar graph represents statistically significant differences on the CS + noise trials on each individual test block (★★ $P<0.05$ relative to the saline group's CS + noise scores on each test block). Clearly, the mean startle scores on the CS + noise trial type for the raclopride and SCH 23390-infused rats were significantly smaller than that obtained from the saline-treated rats across most of the test blocks. This further demonstrates that pretraining intra-BLA raclopride and SCH 23390 prevents CS-induced startle in rats.

Figure 30. The grand mean (S.E.M. ±) of the difference scores ([CS + noise]-[noise-alone]) collapsed over the five fear acquisition test blocks after the intra-basolateral amygdaloid infusion of saline, raclopride (4.0 μg) or SCH 23390 (2.0 μg). The significance level ★ $P<0.05$ is relative to the saline control group (N=13 per experimental group).

Figure 31. Mean (S.E.M. ±) acoustic startle amplitudes and difference score ([4 CS + noise]-[4 noise-alone]) results for the saline, raclopride (4.0 μg) and SCH 23390 (2.0 μg) groups on the retention test conducted 48 hours after bilateral basolateral amygdaloid infusions and 5 fear conditioning/testing blocks (★ $P<0.05$ CS + noise relative to the noise-alone trial type; ✦ $P<0.05$ difference scores of raclopride 4.0 μg or SCH 23390 2.0 μg drug group relative to the saline group's difference score). Each experimental group contained N=13 rats. Double star symbols (★★) placed over the raclopride and SCH 23390 groups denote a statistically significant between-group difference on the CS + noise trial type relative to the saline group (★★ $P<0.05$ relative to the saline group's CS + noise mean startle score on the retention test).

Figure 32. Mean (S.E.M. ±) difference scores ([4 CS + noise]-[4 noise-alone]) of the saline, raclopride (4.0 μg) and SCH 23390 (2.0 μg) groups (N=13 per group) on the retention test administered 48 hours after basolateral amygdaloid infusion and conditioning/testing (★ $P<0.05$ relative to the saline control group).

Figure 33. The mean movement amplitudes (S.E.M. ±) recorded during the presentation of 5 blocks of 8 light + footshock fear conditioning trials to rats that were infused with either saline, raclopride (4.0 μg) or SCH 23390 (2.0 μg) into the basolateral amygdala (N=13 per group).

Figure 34. Mean (S.E.M. ±) acoustic startle amplitude of the last 4 noise-alone trials prior to fear conditioning (Preshock Noise) for saline, raclopride (4.0 μg) or SCH 23390 (2.0 μg) basolateral amygdala infusion groups versus the average startle amplitudes obtained on each of the 5 test blocks containing 4 noise-alone trials presented after light + footshock pairings (depicted as Noise 1 to Noise 5). The symbol (★ $P<0.05$) represents

significant differences of post-shock acoustic startle means (Noise 1 to 5) relative to preshock startle means for all experimental groups (N=13 per group).

Figure 35. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented ad open circles (◎) on the right for the saline control group (N=10) of Experiment 1E that examined the effects of dopamine D_1 amygdaloid receptor antagonism on acoustic startle responding. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 36. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented ad open circles (◎) on the right for the SCH 23390 4.0 μg challenge group (N=11) of Experiment 1E that examined the effects of dopamine D_1 amygdaloid receptor antagonism on acoustic startle responding. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 37. Mean (S.E.M. ±) acoustic startle amplitudes on the pre-infusion and the post-infusion acoustic startle tests for rats infused with either saline (N=10) or the SCH 23390 4.0 μg challenge dose into the basolateral amygdala. The mean difference scores ([post-infusion startle]-[pre-infusion startle]) (S.E.M. ±) demonstrates that infusion of a 4.0 μg challenge dose of SCH 23390 into the basolateral amygdala does not interfere with the capacity of animals to startle in response to a 95 decibel level white-noise burst, indicating normal sensorimotor responding to acoustic stimulus presentations.

Figure 38. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock experimental groups (N=10 each) in the behavioural study that examined the reinstatement of fear-potentiated startle.

Figure 39. Mean white-noise intensity levels (S.E.M. ±) in decibels that were used induce stable acoustic startle responses in the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock experimental groups (N=10 each) that were designed to study the reinstatement of fear-potentiated startle behaviourally.

Figure 40. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 30 light + footshock fear conditioning trials to rats in the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock groups (N=10 each).

Figure 41. Mean (S.E.M. ±) acoustic startle amplitudes on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ P<0.05 relative to noise-alone trials) for the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock groups (N=10 each). Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depicts the equally robust levels of fear-potentiated startle found in all groups 48 hours after 30 fear conditioning trials and before extinction training occurred.

Figure 42. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the equally robust levels of fear-potentiated startle expressed by the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock groups (N=10 each) behavioural experimental groups 48 hours after 30 fear conditioning trials.

Figure 43. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of extinction training versus no extinction training and 5 unsignalled footshocks versus no unsignalled footshocks (★ P<0.05 relative to noise-alone trials). The mean difference scores ([10 CS + noise]-[10 noise-alone]) (S.E.M. ±) demonstrate the impact of 5 unsignalled footshocks on the reinstatement of fear-potentiated startle in rats that received fear extinction training (✦ P<0.05 relative to the Extinction + Shock group and the two No Extinction groups. The behavioural experimental groups are; [Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock groups] N=10 per group. The double star symbol (★★) over the CS + noise trial type of the Extinction + No Shock group denotes a statistically significant between-group difference in mean startle on CS + noise trials relative to the Extinction + Shock and the No Extinction + Shock groups (★★ P<0.05).

Figure 44. Mean difference scores (S.E.M. ±) ([10 CS + noise]-[10 noise-alone]) for the Extinction + Shock, Extinction + No Shock, No Extinction + Shock, and No Extinction + No Shock groups (N=10 each) on the fear-potentiated startle reinstatement test conducted 24 hours after either 5 unsignalled footshock presentations or no footshock presentations (★ P<0.05 relative to the Extinction + Shock group and the two No Extinction groups).

Figure 45. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the phosphate buffered saline (PBS) control group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56

mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 46. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 2.0 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 47. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 4.0 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 48. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 8.0 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 48(a) depicts some digital photographs of guide cannula placements above the basolateral amygdaloid complex. Digital photograph (A) displays guide cannulae tracks above the basolateral amygdaloid complex (approximately -2.30 mm to -2.56 mm posterior to bregma). Also included is photograph (B). The left-half of this photograph exhibits the guide cannula placement above the lateral and basolateral amygdala and the right-half of the photo displays a magnified close-up of this image. Notice the protruding nipple-shaped bump at the end of the guide cannula in the close-up. This is created by the dummy stylet that screws down onto the guide and protrudes 0.25 mm below the end of the guide cannula. During the drug infusion process, the internal infusion cannula extended a full 1.0

mm beyond the end of the guide cannula, thus placing the infusion needle and the drug into the basolateral amygdala.

Figure 49. Mean (S.E.M. ±) baseline acoustic startle amplitudes recorded on the third baselining block for the phosphate buffered saline (PBS) and the three raclopride (2.0, 4.0, and 8.0 μg) groups (N=12 per group).

Figure 50. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that were used to induce stable acoustic startle responding for the phosphate buffered saline (PBS) control group (N=12) and the three raclopride (2.0 μg, 4.0 μg and 8.0 μg) drug groups (N=12 per group).

Figure 51. Mean (S.E.M. ±) movement amplitudes recorded during the administration of 30 light + footshock fear conditioning trials to rats in the phosphate buffered saline group (PBS; N=12) and the three raclopride groups (2.0 μg, 4.0 μg and 8.0 μg; N=12 per group).

Figure 52. Mean (S.E.M. ±) acoustic startle amplitudes obtained on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E. M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) display the equally robust magnitude of fear-potentiated startle found in all groups 48 hours after 30 fear conditioning trials and before extinction training and drug infusion occurred.

Figure 53. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the equally robust levels of fear-potentiated startle found in the PBS (N=12) and raclopride (2.0, 4.0 and 8.0 μg; N=12 each) experimental groups 48 hours after 30 fear conditioning trials and before the extinction training and drug infusion occurred.

Figure 54. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 5 unsignalled footshocks to fear-extinguished rats infused with either phosphate buffered saline (PBS) or raclopride (2.0, 4.0 or 8.0 μg) into the basolateral amygdaloid nucleus (N=12 each).

Figure 55. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of infusion of PBS (N=12) or raclopride (2.0, 4.0 and 8.0 μg; N=12 each) into the basolateral amygdala 24 hours earlier and just prior to 5 unsignalled footshock presentation (★ $P<0.05$ relative to noise-alone trials). The mean difference scores ([10 CS + noise]-[10 noise-alone]) (S.E.M. ±) demonstrate the blocking effect of intra-basolateral amygdaloid raclopride infusion (2.0, 4.0 and 8.0 μg) on the reinstatement of fear-potentiated startle when administered prior to 5 unsignalled reminder footshocks (✦ $P<0.05$ relative to the PBS control group). The double star symbol (★★) over the 8.0 μg raclopride group indicates a statistically significant between-group difference in startle on the CS + noise trial type relative to the PBS group (★★ $P<0.05$ relative to the PBS group's CS + noise mean startle score).

Figure 56. Mean difference scores (S.E.M. ±) ([10 CS + noise]-[10 noise-alone]) for the PBS control and raclopride (2.0, 4.0 and 8.0 μg) groups on the final test to assess the fear-potentiated startle reinstatement when bilateral basolateral amygdaloid infusions had been carried out 24 hours earlier and just prior to the presentation of 5 unsignalled footshocks (★ P<0.05 relative to the PBS control group; N=12 for each group).

Figure 57. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the phosphate buffered saline (PBS) and the 4.0 μg raclopride infusion groups (N=12 each) on the fear-potentiated startle reinstatement test. The star (★) represents the significantly different results between the PBS and raclopride 4.0 μg group when an independent T-test was carried out on the difference score data for these two groups (★ P<0.05 relative to the PBS control group).

Figure 58. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the phosphate buffered saline (PBS) control group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 59. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the SCH 23390 group (N=10) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 60. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that was used to induce stable acoustic startle responses for the phosphate buffered saline (PBS) group (N=12) and the SCH 23390 4.0 μg group (N=10).

Figure 61. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the phosphate buffered (PBS) saline group (N=12) and the SCH 23390 4.0 μg group (N=10).

Figure 62. Mean (S.E.M. ±) movement amplitudes recorded during the last presentation of 30 light + footshock fear conditioning trials to rats in the phosphate buffered saline (PBS) control group (N=12) and the SCH 23390 4.0 μg group (N=10).

Figure 63. Mean (S.E.M. ±) acoustic startle amplitudes for the phosphate buffered saline (PBS) control group (N=12) and the SCH 23390 4.0 μg group (N=10) on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ P<0.05 relative to noise-alone trials). The mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) display the equally robust levels of fear-potentiated startle expressed by both experimental groups 48 hours after 30 fear conditioning trials and before extinction training occurred.

Figure 64. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) for the phosphate buffered saline (PBS) control group (N=12) and the SCH 23390 4.0 μg group (N=10) depicts the nearly equivalent magnitude of fear-potentiated startle exhibited by the experimental groups 48 hours after fear conditioning.

Figure 65. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 5 unsignalled footshocks to fear-extinguished rats infused with either phosphate buffered saline (PBS; N=12) or SCH 23390 (4.0 μg; N=10) into the basolateral amygdaloid complex.

Figure 66. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of infusion of either phosphate buffered saline (PBS; N=12) or 4.0 μgs of SCH 23390 (N=10) into the basolateral amygdala 24 hours earlier and just prior to the presentation of 5 unsignalled footshocks (★ P<0.05 relative to noise-alone trials). The mean difference scores ([10 CS + noise]-[10 noise-alone]) and the corresponding S.E.M. ± values highlight the apparent inability of intra-amygdaloid SCH 23390 infusions to block the reinstatement of fear-potentiated startle.

Figure 67. Mean difference scores (S.E.M. ±) ([10 CS + noise]-[10 noise-alone]) for the phosphate buffered saline (PBS) control group (N=12) and the SCH 23390 4.0 μg drug group (N=10) on the final test used to assess fear-potentiated startle reinstatement when bilateral amygdaloid infusions had been carried out 24 hours earlier and just prior to the presentation of the 5 unsignalled footshocks.

Figure 68. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the phosphate buffered saline (PBS) control group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 69. Schematic depictions of guide cannula locations represented as filled circles

(●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the AP5 1.25 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 70. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the AP5 2.5 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 71. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the phosphate buffered saline (PBS) and the AP5 (1.25 μg and 2.5 μg) experimental groups (N=12; each).

Figure 72. Mean white-noise intensity levels (S.E.M.±) recorded in decibels that was used to induce stable acoustic startle responses for the phosphate buffered saline (PBS) group (N=12) and the two AP5 (1.25 and 2.5 μg) groups (N=12 each).

Figure 73. Mean (S.E.M.±) movement amplitudes recorded during the administration of 30 light + footshock fear conditioning trials to rats in the phosphate buffered saline (PBS) and AP5 (1.25 μg and 2.5 μg) experimental groups (N=12; each).

Figure 74. Mean (S.E.M.±) acoustic startle amplitudes obtained on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) highlight the equally robust levels of fear-potentiated startle found in all groups 48 hours after 30 fear conditioning trials and before extinction training and drug infusion occurred. The experimental groups listed above include, the phosphate buffered saline group (N=12) and two AP5 groups (1.25 μg and 2.5 μg; N=12 each).

Figure 75. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depicts the equally robust levels of fear-potentiated startle found in the PBS (N=12) and AP5 (1.25 μg and 2.5 μg; N=12 each) drug groups 48 hours after 30 fear conditioning trials and before extinction training was initiated.

Figure 76. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 5 unsignalled footshocks to fear-extinguished rats infused with either phosphate buffered saline (PBS) or 1.25 and 2.5 µgs of AP5 into the basolateral amygdaloid complex (N=12 each).

Figure 77. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of infusion of PBS (N=12) and AP5 (1.25 µg and 2.5 µg; N=12 each) into the basolateral amygdala 24 hours earlier and prior to 5 unsignalled footshock presentation (★ P<0.05 relative to noise-alone trials). The mean difference scores ([10 CS + noise]-[10 noise-alone]) (S.E.M. ±) demonstrate the impact of amygdaloidal AP5 infusion on the reinstatement of fear-potentiated startle (✦ P<0.05 relative to the PBS control group). The double star symbols (✭) over the two AP5 groups denote a statistically significant between-group difference in startle on the CS + noise trial type relative to the PBS group (✭ P<0.05 relative to the PBS group's CS + noise mean startle score).

Figure 78. Mean difference scores (S.E.M. ±) ([10 CS + noise]-[10 noise-alone]) for the PBS control and AP5 (1.25 µg and 2.5 µg) groups on the final test to assess the fear-potentiated startle reinstatement when bilateral basolateral amygdaloid infusions had been carried out 24 hours earlier and just prior to the presentation of 5 unsignalled footshocks (★ P<0.05 relative to the PBS control group; N=12 for each group).

Figure 79. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the phosphate buffered saline (PBS) control group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 80. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the CNQX 2.5 µg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 81. Schematic depictions of guide cannula locations represented as filled circles

(●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the CNQX 5.0 μg group (N=12) of the fear-potentiated startle reinstatement study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 82. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the phosphate buffered saline (PBS; N=12) and the CNQX groups (2.5 μg and 5.0 μg; N=12 each).

Figure 83. Mean white-noise levels (S.E.M. ±) recorded in decibels that were used to induce stable acoustic startle responding for the phosphate buffered saline (PBS) group (N=12) and the two CNQX (2.5 μg and 5.0 μg) groups (N=12 each).

Figure 84. Mean (S.E.M. ±) movement amplitudes recorded during the administration of 30 light + footshock fear conditioning trials to rats in the phosphate buffered saline (PBS; N=12) and the CNQX groups (2.5 μg and 5.0 μg; N=12 each).

Figure 85. Mean (S.E.M. ±) acoustic startle amplitudes obtained on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) display the equally robust levels of fear-potentiated startle found in all groups 48 hours after 30 fear conditioning trials and before extinction training occurred. The experimental groups listed above include the phosphate buffered saline group (N=12) and two CNQX groups (2.5 μg and 5.0 μg; N=12 each).

Figure 86. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the equally robust levels of fear-potentiated startle found in the phosphate buffered saline (PBS; N=12) and CNQX (2.5 μg and 5.0 μg; N=12 each) experimental groups 48 hours after 30 fear conditioning trials and before extinction training commenced.

Figure 87. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 5 unsignalled footshocks to fear-extinguished rats infused with either phosphate buffered saline (PBS) or CNQX (2.5 μg and 5.0 μg) into the basolateral amygdaloid complex (N=12 for each group).

Figure 88. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and the 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of infusion of PBS (N=12) or CNQX (2.5 and 5.0 μg; N=12 each) into the basolateral amygdala 24 hours earlier and just prior to the presentation of 5 unsignalled footshocks (★ $P<0.05$ relative to noise-alone trials). The mean difference scores ([10 CS + noise]-[10 noise-alone]) (S.E.M. ±) demonstrate the impact of amygdaloidal CNQX infusion on the

reinstatement of fear-potentiated startle (✦ P<0.05 relative to the PBS control group). The double star symbols (✯) over the two CNQX drug groups denote a statistically significant between-group difference in startle on the CS + noise trial type relative to the PBS group (✯ P<0.05 relative to the PBS group's CS + noise mean startle score).

Figure 89. Mean difference scores (S.E.M. ±) ([10 CS + noise]-[10 noise-alone]) for the PBS control and the CNQX (2.5 and 5.0 μg) groups on the final test to assess fear-potentiated startle reinstatement when bilateral basolateral amygdaloid infusions had been carried out 24 hours earlier and just prior to the presentation of 5 unsignalled footshocks (★ P<0.05 relative to the PBS control group; N=12 for each experimental group).

Figure 90. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the phosphate buffered saline (PBS) control group (N=7) of the expression control study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 91. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the raclopride 8.0 μg group (N=7) of the expression control study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 92. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the AP5 1.25 μg group (N=8) of the expression control study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 93. Schematic depictions of guide cannula locations represented as filled circles

(●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the CNQX 2.5 μg group (N=7) of the expression control study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 94. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (◎) on the right for the CNQX 5.0 μg group (N=8) of the expression control study. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 95. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), CNQX 2.5 μg (N=7), and CNQX 5.0 μg (N=8) drug groups of the expression control experiment.

Figure 96. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that were used to induce stable acoustic startle responses for the phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), CNQX 2.5 μg (N=7), and CNQX 5.0 μg (N=8) drug groups of the expression control experiment.

Figure 97. Mean (S.E.M. ±) movement amplitudes recorded in response to footshocks presented during the administration of 30 light + footshock fear conditioning trials to rats belonging to the phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), CNQX 2.5 μg (N=7), and CNQX 5.0 μg (N=8) drug groups of the expression control experiment.

Figure 98. Mean (S.E.M. ±) acoustic startle amplitudes for the phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), CNQX 2.5 μg (N=7), and CNQX 5.0 μg (N=8) drug groups on 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). The mean difference scores (S.E.M. ±) of the pretest ([3CS + noise]-[3 noise-alone]) depict the nearly equivalent and robust magnitude of fear-potentiated startle expressed by the drug groups of the expression control study 48 hours after Pavlovian fear conditioning.

Figure 99. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depicts the nearly equivalent and robust levels of fear exhibited by the phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), CNQX 2.5 μg (N=7), and CNQX 5.0 μg (N=8) drug groups of the expression control study 48 hours after Pavlovian fear conditioning.

Figure 100. Mean (S.E.M. ±) movement amplitudes recorded in response to the presentation of 5 unsignalled footshocks to non-extinguished rats of the expression control study after bilateral infusion of phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), and CNQX (2.5 μg; N=7 and 5.0 μg; N=8 respectively), into the basolateral amygdaloid complex.

Figure 101. Mean (S.E.M. ±) acoustic startle amplitudes for non-fear-extinguished rats of the expression control study on the 10 noise-alone and 10 CS + noise trials of the final fear-potentiated startle test as a function of infusion of phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), and CNQX (2.5 μg ;N=7 and 5.0 μg; N=8) into the basolateral amygdaloid complex 24 hours earlier and just prior to 5 unsignalled footshock presentation (★ P<0.05 relative to noise-alone trials). With the exception of CNQX, the mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) of the final test demonstrate that intra-amygdaloid infusions of PBS, raclopride, and AP5 24 hours before final testing does not diminish the capacity of non-fear-extinguished rats to express fear to a specific CS.

Figure 102. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) on the final test for non-fear-extinguished expression control study rats that received bilateral intra-amygdaloid infusions of phosphate buffered saline (PBS; N=7), raclopride 8.0 μg (N=7), AP5 1.25 μg (N=8), and CNQX (2.5 μg and 5.0 μg; N=7 and N=8 respectively) and 5 unsignalled footshocks 24 hours before testing for the expression of fear-potentiated startle responding.

Figure 103. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for No Extinction + No Unsignalled footshock CNQX 5.0 μg (N=8) control group that was to further evaluate the effects of CNQX basolateral amygdala infusions on the expression of fear-potentiated startle in the absence of unsignalled footshocks. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 104. Mean (S.E. M.±) startle amplitudes for the CNQX 5.0 μg (N=8) No Extinction + No Unsignalled Shock experimental control group on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ P<0.05 relative

to the noise-alone trials). The mean (S.E.M. ±) difference scores ([3 CS + noise]-[3 noise-alone]) of the pretest demonstrates the robust level of fear exhibited by the CNQX 5.0 μg No Extinction + No Unsignalled Shock experimental control group 48 hours after fear conditioning.

Figure 105. Mean (S.E.M. ±) startle amplitudes for the CNQX 5.0 μg No Extinction + No Unsignalled Shock experimental control group (N=8) on the 10 noise-alone and 10 CS + noise trials of the final test conducted 24 hours after intra-amygdaloid infusion of CNQX (5.0 μg) only (★ P<0.05 relative to noise-alone trials). The mean (S.E.M. ±) of the difference score ([10 CS + noise]-[10 noise-alone]) depicts the robust magnitude of fear exhibited by the CNQX 5.0 μg control group (N=8) on the final test when no unsignalled footshocks were administered after amygdaloidal CNQX infusion had been made.

Figure 106. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) on the final test for the non-extinguished + 5 unsignalled footshock groups from Experiment 4 (PBS N=7; raclopride 8.0 μg N=7; AP5 1.25 μg N=8) versus the mean difference score of the CNQX 5.0 μg No Extinction + No 5 Shock group (N=8) of Experiment 5. It is important to note that ANOVA of the difference score data yielded a non-significant result, indicating that all groups represented in the above figure exhibited roughly equivalent levels of conditioned fear as measured through the potentiation of acoustic startle responding.

Figure 107. Mean (S.E.M. ±) acoustic startle amplitudes on 10 noise-alone and 10 CS + noise trials of the final test for the non-extinguished + 5 unsignalled shock drug groups of Experiment 4 that expressed fear-potentiated startle 24 hours after intra-amygdalar infusion of PBS (N=7), raclopride (8.0 μg; N=8) or AP5 (1.25 μg; N=8) are shown on the left under the 5 unsignalled Shocks title. On the extreme right are the final test results for the CNQX 5.0 μg No Unsignalled Shock group of Experiment 5 along with the mean (S.E.M. ±) acoustic startle amplitude on 10 noise-alone and 10 CS + noise trials. The mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) of the final test indicate that the CNQX rats of Experiment 5 exhibited a magnitude of fear similar to that expressed by certain groups in Experiment 4. It is noteworthy that all groups depicted in the above figure exhibited robust FPS (★ P<0.05 relative to noise-alone trials). However, the CNQX 5.0 μg group did show a general depression in acoustic startle responding to both types of stimulus presentation.

Figure 108. Schematic depictions of guide cannula locations represented as filled circles (●) on the left and the approximate location of infusion needles represented as open circles (○) on the right for the 2.5 μg AP5 Ataxia Control group (N=7) of Experiment 6. Guide cannulae were implanted 1.0 mm above the medial portion of the basolateral amygdaloid nucleus (coordinates A.P -2.80 mm from bregma, M.L. ± 4.80 mm from the midline sagittal suture, D.V. -7.40 mm from the skull surface). The infusion needles extended 1.0 mm ventrally beyond the locations of the guide cannula. Representative sections (top to bottom; -1.80 m, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 109. Mean (S.E.M. ±) baseline startle amplitudes before (Pre-Drug) and after (Post-Drug) the infusion of 2.5 μg of AP5 into the basolateral amygdaloid complex. The mean (S.E.M. ±) difference score ([Post-drug startle]-[Pre-drug startle]) is depicted by the third bar of the graph and clearly indicated that there were no significant differences between predrug and post-drug acoustic startle amplitudes.

Figure 110. The above figure schematically depicts the location of bipolar electrodes in the basolateral and central lateral amygdaloid nuclear groups and is represented by the solid filled circles (●). On the left are the approximate location of implanted electrodes for the Extinction + Stimulation group (N=12) and on the right are the electrode placements for the Extinction + No Stimulation group (N=12) of Experiment 7A. Bipolar electrodes were unilaterally implanted into either the left or right basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L.± 4.80 mm from the midline sagittal suture, D.V. 8.60 mm from the skull surface). Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 111. The above figure schematically depicts the location of bipolar electrodes in the basolateral and central lateral amygdaloid nuclear groups and is represented by the solid filled circles (●). On the left are the approximate location of implanted electrodes for the No Extinction + Stimulation group (N=11) and on the right are the electrode placements for the No Extinction + No Stimulation group (N=12) of Experiment 7A. Bipolar electrodes were unilaterally implanted into either the left or right basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L.± 4.80 mm from the midline sagittal suture, D.V. 8.60 mm from the skull surface). Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 112. The above figure schematically depicts the location of bipolar electrodes in the basolateral and central lateral amygdaloid nuclear groups and is represented by the solid filled circles (●). On the left are the approximate location of implanted electrodes for the Extinction + In Context Stimulation group (N=12) and on the right are the electrode placements for the Extinction + Out of Context Stimulation group (N=12) of Experiment 7B. Bipolar electrodes were unilaterally implanted into either the left or right basolateral amygdaloid nucleus (coordinates A.P. -2.80 mm from bregma, M.L.± 4.80 mm from the midline sagittal suture, D.V. 8.60 mm from the skull surface). Representative sections (top to bottom; -1.80 mm, -2.12 mm, -2.30 mm, -2.56 mm, -2.80 mm, and -3.14 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 113 (a). These two digital photographs of a rat brain clearly depict the location of a bipolar electrode in the basolateral amygdala (coronal sections were taken approximately -1.80 mm to -2.12 mm posterior to bregma). Notice the presence of the electrode tip near

the middle of the basolateral amygdaloid complex (photo slides A and B) as denoted by the small dark arrows.

Figure 113 (b). These two digital photographs also depict the placement of a bipolar electrode in the basolateral amygdala (coronal sections were taken approximately -2.12 mm to -2.30 mm posterior to bregma). Again, notice the presence of the electrode tip near the middle of the basolateral amygdaloid complex (photo slides C and D) as denoted by the small dark arrows.

Figure 113 (c). These two digital photographs are magnified close-ups of photo-slides A and B from Figure 113 (a). These close-up photographs clearly demonstrate that the non-insulated portion of the bipolar electrode was located in the basolateral amygdala.

Figure 113 (d). These two digital photographs are magnified close-ups of photo-slides C and D from Figure 113 (b). Again notice that these close-up photographs clearly demonstrate that the non-insulated portion of the bipolar electrode tips were located in the basolateral amygdala.

Figure 113 (e). The digital photograph (A) of another rat clearly shows the location of a bipolar electrode in the basolateral amygdala (coronal sections taken at -2.30 mm to -2.56 mm posterior to bregma). The small dark arrow in the photograph points to the location of the electrode tip (photo A). Digital photograph (B) is a magnified version of photograph A. As can be seen the bipolar electrode tip in this rat was clearly in the middle of the basolateral amygdaloid complex.

Figure 114. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) groups.

Figure 115. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that was used to induce stable acoustic startle responses in the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) groups.

Figure 116. Mean (S.E.M. ±) movement amplitudes recorded during the presentation of 30 light + footshock fear conditioning trials to rats in the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) experimental groups.

Figure 117. Mean (S.E.M. ±) acoustic startle amplitudes on the 3 noise-alone and 3 CS + noise trials of the pretest for the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) groups as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3

noise-alone]) depicts the equivalent levels of fear-potentiated startle exhibited by all groups 48 hours after 30 fear conditioning trials.

Figure 118. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depicts the equally robust magnitude of fear-potentiated startle responding expressed by the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) experimental groups 48 hours after exposure to 30 fear conditioning trials.

Figure 119. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of extinction or no extinction training and electrical stimulation of the basolateral amygdala or no stimulation (★ $P<0.05$ relative to noise-alone trials). The mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) groups demonstrate the impact of electrical stimulation of the basolateral amygdala 24 hours earlier on the reinstatement of fear-potentiated startle during the final test (✦ $P<0.05$ relative to the Extinction + BLA Stimulation and the two No Extinction groups).

Figure 120. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) trials for the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) groups on the final test to assess the reinstatement of fear-potentiated startle 24 hours after rats either received 100 stimulations of the basolateral amygdaloid complex (BLA) or no BLA stimulations (★ $P<0.05$ relative to the Extinction + BLA Stimulation group and the two No Extinction experimental groups).

Figure 121. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the Extinction + In Context BLA Stimulation and the Extinction + Out of Context BLA Stimulation groups (N=12; each).

Figure 122. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that was used to induce stable acoustic startle responses in the Extinction + In Context BLA Stimulation (N=12) and the Extinction + Out of Context BLA Stimulation (N=12) groups.

Figure 123. Mean (S.E.M. ±) movement amplitude recorded during the presentation of 30 light + footshock fear conditioning trials to rats in the Extinction + In Context BLA Stimulation and Extinction + Out of Context BLA Stimulation experimental groups (N=12; each).

Figure 124. Mean (S.E.M. ±) acoustic startle amplitudes on the 3 noise-alone and 3 CS + noise trials of the pretest for the Extinction + In Context BLA (N=12) and the Extinction + Out of Context BLA (N=12) groups as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E.M. ±) of the pretest ([3 CS +

noise]-[3 noise-alone]) depicts the equivalent levels of fear-potentiated startle exhibited by both experimental groups 48 hours after 30 fear conditioning trials.

Figure 125. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depicts the equally robust magnitude of fear-potentiated startle expressed by both the Extinction + In Context BLA Stimulation and the Extinction + Out of Context BLA Stimulation groups (N=12; each) 48 hours after exposure to 30 fear conditioning trials.

Figure 126. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of extinction training and in context versus out of context electrical stimulation of the basolateral amygdala (★ P<0.05 relative to noise-alone trials). The mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the Extinction + In Context BLA Stimulation (N=12) and the Extinction + Out of Context BLA Stimulation groups demonstrate the impact of electrical stimulation of the basolateral amygdala on fear-potentiated startle reinstatement. The results also highlight the important role played by contextual factors in influencing the magnitude of fear reinstatement exhibited by the two experimental groups (✦ P<0.05 relative to the Extinction + In Context BLA Stimulation group).

Figure 127. Mean (S.E.M.±) difference scores ([10 CS + noise]-[10 noise-alone]) for the Extinction + In Context BLA Stimulation (N=12) and the Extinction + Out of Context BLA Stimulation (N=12) groups on the final test to assess the reinstatement of fear-potentiated startle 24 hours after rats received 100 electrical stimulations of the basolateral amygdaloid complex either in the fear conditioning/testing apparatus (In Context) or in a completely different apparatus (Out of Context). The significant differences between the two groups highlights the importance of contextual cues and fear memories on the reinstatement of fear responding (★ P<0.05 relative to the Extinction + In Context BLA Stimulation group).

Figure 128. Mean (S.E.M. ±) after discharge current thresholds recorded from the amygdala of rats in the Extinction + BLA Stimulation (N=12), Extinction + No Stimulation (N=12), No Extinction + BLA Stimulation (N=11) and No Extinction + No Stimulation (N=12) experimental groups (★ P<0.05 relative to the Extinction + BLA Stimulation group only).

Figure 129 depicts a typical after-discharge pattern recorded from neurons in the basolateral complex after electrical stimulation was used to induce after-discharge activity in the amygdala. To the left of the dark arrow is the cellular activity of the amygdala prior to the after-discharge inducing electrical stimulation and just to the right of the dark arrow is the characteristic after-discharge pattern recorded from the amygdala. As can be seen the after-discharge activity in the amygdala generally produces numerous spikes that are high in amplitude and are characterized by short inter-spike intervals. Further to the right of the after-discharge pattern neurons have returned to normal activity levels which indicates that they are either in a refractory period or that they have returned back to their membrane resting potentials. The first two recordings (1 and 2) were obtained from two rats that belonged to the Extinction + Basolateral Amygdala Stimulation and the No Extinction +

Basolateral Amygdala Stimulation groups of Experiment 7A, whilst the third and fourth recordings were obtained from rats belonging to the Extinction + No Stimulation group of Experiment 7A.

Figure 130 schematically depicts the location of bipolar electrodes in the granular, dysgranular, and posterior agranular insular cortices bordering the ectorhinal and perirhinal cortices. These electrode locations are represented by the solid filled circles (●). On the left are the approximate location of implanted electrodes for the Extinction + Perirhinal/Insular Cortex Stimulation group (N=8) and on the right are the electrode placements for the Extinction + Perirhinal/Insular No Stimulation control group (N=9) of Experiment 8. Bipolar electrodes were unilaterally implanted into either the left or right Perirhinal/Insular cortex region of the temporal lobe (coordinates A.P. -2.90 mm from bregma, M.L. ± 6.40 mm from the midline sagittal suture, D.V. -6.80 mm from the skull surface). Representative sections (top to bottom; -1.80 mm, -2.12 mm,-2.30 mm, -2.56 mm and -2.80 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1998.

Figure 131. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the Perirhinal/Insular Extinction + Stimulation (N=8) and the Perirhinal/Insular Cortex Extinction + No Stimulation (N=9) groups.

Figure 132. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that were used to induce stable acoustic startle responses in animals belonging to the Perirhinal/Insular Cortex Extinction + Stimulation (N=8) and Perirhinal/Insular Cortex Extinction + No Stimulation (N=9) groups.

Figure 133. Mean (S.E.M. ±) movement amplitudes recorded in response to footshocks administered during the presentation of 30 light + footshock fear conditioning trials to rats in the Perirhinal/Insular Cortex Extinction + Stimulation (N=8) group and the Perirhinal/Insular Cortex Extinction + No Stimulation (N=9) control group.

Figure 134. Mean (S.E.M. ±) acoustic startle amplitudes for the Perirhinal/Insular Cortex Extinction + Stimulation (N=8) and Perirhinal/Insular Cortex Extinction + No Stimulation (N=9) groups on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ $P<0.05$ relative to noise-alone trials). Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the nearly equivalent levels of fear-potentiated startle exhibited by both experiment groups 48 hours after exposure to 30 light + footshock fear conditioning trials.

Figure 135. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the near equal and robust levels of fear exhibited by the Perirhinal/Insular Cortex Extinction + Stimulation (N=8) and the Perirhinal/Insular Cortex Extinction + No Stimulation (N=9) groups 48 hours after fear conditioning.

Figure 136. Mean (S.E.M. ±) acoustic startle amplitudes on the 10 noise-alone and 10 CS + noise trials of the final test for fear-potentiated startle reinstatement as a function of

Perirhinal/Insular cortex stimulation or no stimulation 24 hours earlier (★ P<0.05 relative to noise-alone trials). The mean (S.E.M. ±) difference scores of the final test ([10 CS + noise]-[10 noise-alone]) for the Perirhinal/Insular Cortex Extinction + Stimulation group (N=8) and the Perirhinal/Insular Cortex Extinction + No Stimulation group (N=9) demonstrate the impact on fear reinstatement that occurred as a result of electrical stimulation of the perirhinal and insular cortical areas 24 hours earlier (✦ P<0.05 relative to the Perirhinal/Insular Cortex Extinction + Stimulation group mean difference score).

Figure 137. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) of the final test for the Perirhinal/Insular Cortex Extinction + Stimulation group (N=8) and the Perirhinal/Insular Cortex + No Stimulation group (N=9) twenty-four hours after either stimulation of the perirhinal and insular cortical regions or no stimulation.

Figure 138. Mean (S.E.M. ±) percentage increase of CS + noise over noise-alone trials for the Perirhinal/Insular Cortex Extinction + Stimulation group (N=8) and the Perirhinal/Insular Cortex Extinction + No Stimulation group (N=9) based on the formula: Percent Increase or Decrease = ([Larger startle score ÷ Smaller startle score]-1) x 100 (★ P<0.05 relative to the Perirhinal/Insular Cortex Extinction + Stimulation group's percent increase score).

Figure 138(a). Mean (S.E.M. ±) acoustic startle amplitudes on 10 noise-alone and 10 CS + noise trials for the final test for fear-potentiated startle reinstatement as a function of electrical stimulation of either the anterior Insula region (N=4) or the posterior Insula/Ectorhinal region (N=4) (★ P<0.05 relative to noise-alone trials). As can be seen only the anterior Insula electrode Extinction + Stimulation group exhibited significant CS-induced startle on the FPS reinstatement test. The mean (S.E.M. ±) difference scores of the final test ([10 CS + noise]-[10 noise-alone]) for the anterior Insula electrode group and the posterior Insula/Ectorhinal electrode group were found not to be significantly different from each other. Also, the two experimental groups depicted above did not differ from each other on the percent increase in startle from noise-alone to CS + noise trials. However, by visually inspecting the graphs above, it does seem as though stimulation of the anterior insula region did generally have a more positive impact on FPS reinstatement than insula/ectorhinal stimulation.

Figure 139. Mean (S.E.M. ±) after discharge current thresholds for the Perirhinal/Insular Cortex Extinction + Stimulation group (N=7) and the Perirhinal/Insular Cortex Extinction + No Stimulation control group (N=9).

Figure 140 depicts a typical after-discharge pattern recorded from neurons in the perirhinal/insular cortical region of the temporal lobe after electrical stimulation was used to induce after-discharge activity in this region. The dark arrow represents the point at which the electrical stimulation was administered to the perirhinal/insular cortical region. To the left of the dark arrow is the cellular activity of perirhinal/insular cortex neurons prior to the after-discharge inducing electrical stimulation and just to the right of the dark arrow is the characteristic after-discharge pattern recorded from the insular and perirhinal cortical area. As can be seen the after-discharge activity in the perirhinal/insular cortices of

the temporal lobe generally produces more frequent spikes that are as high or slightly higher in amplitude that those recorded from the amygdala during after-discharge activity. The perirhinal/insular cortex after discharge pattern is also characterized by short inter-spike intervals, however, the frequency of spikes seems to occur more rapidly that those in the amygdala. Further to the right of the after-discharge pattern the neurons exhibit an uncharacteristic quiet period lasting several seconds (perhaps indicative of a refractory period) followed by intermittent large spikes separated by smaller spiking activity. It is important to point out that the length of this quiet period varied from animal to animal and sometimes animals exhibited significant neuronal activity after the quiet period. The firs two after-discharge recording patterns presented above were obtained from rats that belonged to the Extinction + Perirhinal/Insular cortex Stimulation group of Experiment 8. The third recording comes from an animal belonging to the Extinction + No Stimulation Perirhinal/Insular cortex control group of Experiment 8.

Figure 141. A schematic depiction of the bipolar electrodes located in or near the anterior portions of the dorsal periaqueductal gray (dPAG) for the Extinction + dPAG Stimulation group (N=11). The electrode locations are represented by the solid filled circles (●). Bipolar electrodes were unilaterally implanted into either the left or right dPAG nuclei (coordinates A.P. -6.30 mm from bregma, M.L. ± 0.30 mm from the midline sagittal suture, D.V. 5.00 mm from the skull surface). Representative sections (from top to bottom; -5.20 mm, -5.30 mm, -5.60 mm, -5.80 mm, -6.04 mm and 6.80 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson.

Figure 142. A schematic depiction of the bipolar electrodes located in or near the ventral tegmental area (VTA) for the Extinction + VTA Stimulation group (N=11). The electrode locations are represented by the solid filled circles (●). Bipolar electrodes were unilaterally implanted into either the left or right VTA nuclear group (coordinates A.P. -4.80 mm from bregma, M.L. ± 0.80 mm from the midline sagittal suture, D.V. -8.20 mm from the skull surface). Representative sections (top to bottom; -4.16 mm, -4.30 mm, -4.52 mm, -4.80 mm and -5.20 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 143. A schematic depiction of the bipolar electrodes located in or near the ventral tegmental area (VTA; N=6) on the left and anterior portions of the dorsal periaqueductal gray (dPAG; N=5) on the right for the Mid-Brain Extinction + No Stimulation Control group (N=11). This Mid-Brain Extinction + No Stimulation group (N=11) served as the no stimulation control group for the Extinction + VTA Stimulation and Extinction + dPAG Stimulation groups (N=11; each). The electrode locations are represented by the solid filled circles (●) and all and bipolar electrodes were unilaterally implanted into either the left or right VTA and dPAG (VTA coordinates A.P. -4.80 mm from bregma, M.L. ± 0.80 mm from the midline sagittal suture, D.V. -8.20 mm from the skull surface; dPAG coordinates A.P. -6.30 mm from bregma, M.L. ± 0.30 mm from the midline sagittal suture, D.V. 5.00 mm from the skull surface). Representative sections (Left side: from top to bottom VTA; -4.30 mm, -4.52 mm and -4.80 mm from bregma; Right side: top to bottom dPAG; -5.20 mm, -5.30 mm, and 5.60 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 144. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the mid-brain + No Stimulation , rostrodorsal periaqueductal gray + Stimulation and the ventral tegmental are + Stimulation experimental groups (N=11 per group).

Figure 145. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that were used to induce stable acoustic startle responses in the Extinction + Midbrain No Stimulation group (N=11), the rostrodorsal Periaqueductal Gray + Stimulation group (N=11) and the ventral tegmental area Stimulation group (N=11) respectively.

Figure 146. Mean (S.E.M. ±) movement amplitudes recorded in response to footshock presentation during the administration of 30 light + footshock fear conditioning trials to rats of the mid-brain Extinction + No Stimulation group (N=11), the rostrodorsal periaqueductal gray Extinction + Stimulation group (N=11), and the ventral tegmental area Extinction + Stimulation group (N=11).

Figure 147. Mean (S.E.M. ±) acoustic startle amplitudes for the mid-brain Extinction + No Stimulation, rostrodorsal periaqueductal gray Extinction + Stimulation and ventral tegmental area Extinction + Stimulation groups (N=11 per group) on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ P<0.05 relative to noise-alone trials). The mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the nearly equivalent magnitude of fear-potentiated startle exhibited by all three experimental groups 48 hours after 30 fear conditioning trials were administered to each group.

Figure 148. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) depict the equally robust levels of fear exhibited by all three experimental groups ([mid-brain Extinction + No Stimulation group; N=11] [rostrodorsal periaqueductal gray Extinction + Stimulation group; N=11] [ventral tegmental area Extinction + Stimulation group; N=11]) forty-eight hours after fear conditioning.

Figure 149. Mean (S.E.M. ±) acoustic startle amplitudes for the mid-brain Extinction + No Stimulation group (N=11), rostrodorsal periaqueductal gray Extinction + Stimulation group (N=11) and ventral tegmental area Extinction + Stimulation group (N=11) on the 10 noise-alone and 10 CS + noise trials of the fear-potentiated startle reinstatement test as a function of stimulation or no stimulation that occurred 24 hours earlier (★ P<0.05 relative to the noise-alone trials). The mean difference scores (S.E.M. ±) of the reinstatement test ([10 CS + noise]-[10 noise-alone]) depict the effect of electrical stimulation of the periaqueductal gray and ventral tegmental area on the magnitude of fear-potentiated startle reinstatement exhibited (✦ P<0.05 relative to the VTA Extinction + Stimulation group only).

Figure 150. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the mid-brain Extinction + No Stimulation, rostrodorsal periaqueductal gray Extinction + Stimulation, and ventral tegmental area Extinction + Stimulation groups (N=11 per group) on the fear-potentiated startle test carried out 24 hours after electrical stimulation or no

stimulation of the rostrodorsal PAG and ventral tegmental area (★ P<0.05 relative to the Extinction + VTA Stimulation group only).

Figure 151. Mean (S.E.M. ±) acoustic startle amplitudes for the mid-brain Extinction + No Stimulation Control group (N=11) and the Extinction + VTA Stimulation group (N=11) on the 10 noise-alone and 10 CS + noise trials of the fear-potentiated startle reinstatement test as a function of either stimulation or no stimulation that occurred 24 hours earlier (★ P<0.05 relative to noise-alone trials). The mean (S.E.M. ±) difference scores of the reinstatement test ([10 CS + noise]-[10 noise-alone]) depict the effect of electrical stimulation of the ventral tegmental area (VTA) on the magnitude of fear-potentiated startle reinstatement expressed by this group when it is compared to the mid-brain Extinction + No Stimulation controls (✦ P<0.05 relative to the Extinction + VTA Stimulation group).

Figure 152. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the mid-brain Extinction + No Stimulation and Extinction + ventral tegmental area (VTA) Stimulation groups on the fear-potentiated startle reinstatement test carried out 24 hours after either electrical stimulation of the VTA or no stimulation of the mid-brain control group (N=11 per experimental group; ★ P<0.05 relative to the Extinction + VTA Stimulation group).

Figure 153. A schematic depiction of the bipolar electrodes located in or near the caudal portions of the ventral periaqueductal gray (vPAG). The electrode locations are represented by solid filled circles (●). On the left are the electrode locations for the Extinction + vPAG Stimulation group (N=5) while on the right are the electrode locations for the Extinction + vPAG No Stimulation control group (N=7). Bipolar electrodes were unilaterally implanted into either the left or right vPAG nuclei for both experimental groups (coordinates A.P. -7.80 mm from bregma, M.L. ± 0.70 mm from the midline sagittal suture, D.V. -5.80 mm from the skull surface). Representative sections (top to bottom; -6.80 mm, -7.04 mm, -7.30 mm, -7.64 mm and -7.80 mm from bregma) were taken from *The Rat Brain in Stereotaxic Coordinates,* by G. Paxinos and C. Watson, 1986.

Figure 154. Mean (S.E.M. ±) baseline startle amplitudes recorded on the third baselining block for the ventral periaqueductal gray Extinction + No Stimulation group (N=7) and the ventral periaqueductal gray Extinction + Stimulation group (N=5).

Figure 155. Mean white-noise intensity levels (S.E.M. ±) recorded in decibels that was used to induce stable acoustic startle responses for the ventral periaqueductal gray Extinction + No Stimulation group (N=7) and the ventral periaqueductal gray Extinction + Stimulation group (N=5).

Figure 156. Mean (S.E.M. ±) movement amplitudes recorded in response to footshocks presented during the administration of 30 light + footshock fear conditioning trials to ventral periaqueductal gray Extinction + Stimulation (N=5) and the ventral periaqueductal gray Extinction + No Stimulation (N=7) experimental groups.

Figure 157. Mean (S.E.M. ±) acoustic startle amplitudes for the ventral periaqueductal gray Extinction + No Stimulation (N=7) and Extinction + Stimulation (N=5) groups on the 3 noise-alone and 3 CS + noise trials of the pretest as a function of fear conditioning only (★ P<0.05 relative to the noise-alone trials). The mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) display the equally robust magnitude of fear expressed by the two experimental groups 48 hours after Pavlovian fear conditioning.

Figure 158. Mean difference scores (S.E.M. ±) of the pretest ([3 CS + noise]-[3 noise-alone]) for the ventral periaqueductal gray Extinction + No Stimulation (N=7) and Extinction + Stimulation (N=5) experimental groups depicts the nearly equivalent and robust levels of fear exhibited by both groups 48 hours after exposure to 30 light + footshock fear conditioning trials.

Figure 159. Mean (S.E.M. ±) acoustic startle amplitude for the ventral periaqueductal gray Extinction + No Stimulation (N=7) and Extinction + Stimulation (N=5) experimental groups on the 10 noise-alone and 10 CS + noise trials of the test designed to measure the reinstatement of fear-potentiated startle as a function of electrical stimulation of the ventral periaqueductal gray 24 hours earlier (★ P<0.05 relative to noise-alone trials). The mean difference scores (S.E.M. ±) of the final test ([10 CS + noise]-[10 noise-alone]) show the effect of electrical stimulation of the ventral periaqueductal gray on the reinstatement of fear-potentiated startle in rats that were exposed to fear-extinction training.

Figure 160. Mean (S.E.M. ±) difference scores ([10 CS + noise]-[10 noise-alone]) for the ventral periaqueductal gray Extinction + No Stimulation (N=7) and Extinction + Stimulation (N=5) groups on the final test conducted 24 hours after either electrical stimulation or no electrical stimulation of the caudal portions of the ventral periaqueductal gray.

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Abbreviations

Act-D	actinomycin-D
AD	After-discharge
AHP	After hyperpolarization shift
α	Alpha
AMPA	Alpha-amino-3-hydroxy-methylisoxazole-4 -proprionic acid
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
AAA	Anterior Amygdaloid Area
AHA	Amygdalo Hippocampal Area
AESOP	Affective extension sometimes-opponent-process
AP5	amino-5-phophonovaleric acid
AP7	amino-7-phosphonoheptanoic acid
AStr	Amygdalostriatal transition area
β	Beta
BL	Basolateral nucleus
BLA	Basolateral Amygdala
BMA	Basomedial Amygdala (or) Basomedial amygdaloid nucleus
BNST	Bed Nucleus of the Stria Terminalis
md-BNST	mediodorsal Bed Nucleus of the Stria Terminalis
BNST (Ld)	lateral and dorsal subdivision of the Bed Nucleus of the Stria Terminalis

BNST (m&L)	medial and lateral divisions of the Bed Nucleus of the Stria Terminalis
cVAF	caudal ventral amygdalofugal pathway
CGRP	calcitonin gene-related peptide
Ca^{2+}	Calcium
αCaMKII	alpha-Ca^{2+}/ calmodulin dependent kinase II
Ce	Central amygdaloid nuclei
CeA	Central amygdala
CCK	Cholecytokinin
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione disodium
Cl^-	Chloride
CLC	lateral capsular subdivision of the central amygdaloid nucleus
CoA	anterior cortical amygdaloid nucleus
CoM	medial cortical amygdaloid nucleus
CS	Conditioned Stimulus
CS2	secondary Conditioned Stimulus
CRF	corticotropin-releasing factor
CRH	corticotropin-releasing hormone
cAMP	cyclic adenosine monophosphate
CRE	cAMP response-element
CREB	cAMP response-element binding protein
CPL1	cytoplasmic loop 1
CPL2	cytoplasmic loop 2

CPL3	cytoplasmic loop 3
DA	Dopamine
DMCM	methyl-6,-7-dimethoxy-4-ethyl-beta-carboline-3-Carboxylate
DOPAC	3, 4-dihydroxyphenylacetic acid
D-AP5	D-2-amino-5-phosphonovaleric acid
DNA	Deoxyribonucleic acid
DNQX	6,7-dinitroquinoxaline-2,3-dione
EEG	Electroencephalographic
EMG	Electromyographic
E-LTP	Early Long-Term-Potentiation
EPSC	Excitatory postsynaptic current
EPSCs	Excitatory postsynaptic currents
EPSP	Excitatory postsynaptic potential
EPSPs	Excitatory postsynaptic potentials
ERK	Extra-cellular regulated kinase
FPS	Fear-Potentiated Startle
γ	Gamma
GABA	Gamma-aminobutyric acid
GAD	Glutamate acid decarboxylase
H7	1-(5-isoquinolinesulfonyl)-2-methylpiperazine
HRP	Horse radish peroxidase
HSVm-CREBt	Herpes simplex vector-mediated CREB transfer
HVA	Homovanillic acid

Hz	Hertz
ICV	Intracerebroventricular
IL	Infralimbic cortex
IPSC	Inhibitory postsynaptic current
IPSCs	Inhibitory postsynaptic currents
IPSP	Inhibitory postsynaptic potential
IPSPs	Inhibitory postsynaptic potentials
IP_3	inositol, 1, 4, 5-triphosphate
ISI	inter-stimulus interval
ITI	inter-trial interval
La-AStr	lateral amygdala-amygdalostriatal pathway
La-BL	lateral amygdala-basolateral nuclear pathway
LA	lateral amygdaloid nucleus (or) lateral nucleus of the amygdala
LAd	dorsal subdivision of the lateral amygdaloid nucleus
LC	locus coeruleus
LGN	lateral geniculate nucleus of the thalamus
L.H.	lateral hypothalamus
LPN	lateral posterior nucleus of the thalamus
LTD	Long-Term-Depression
LTM	Long-Term-Memory
LTP	Long-Term-Potentiation
L-LTP	Late-Long-Term-Potentiation
MAPK	mitogen-activated protein kinase

Mg^{2+}	Magnesium
mGluR	metabotropic glutamate receptor
mGluRs	metabotropic glutamate receptors
mGluR1	metabotropic glutamate receptor 1
mGluR5	metabotropic glutamate receptor 5
MGN	Medial Geniculate nucleus of the thalamus
mdMGN	medial division of the Medial Geniculate nucleus of the thalamus
MFB	medial forebrain bundle
mRNA	messenger ribonucleic acid
mPFC	medial Prefrontal Cortex
NE	norepinephrine
NLOT	Nucleus of the lateral olfactory tract
NMDA	N-Methyl-D-Aspartate
Nuc Acc	Nucleus Accumbens
6-OHDA	6-hydroxy-dopamine
O1	extracellular loop 1
O2	extracellular loop 2
O3	extracellular loop 3
Oc1	primary (occipital) visual cortex
Oc2	secondary (occipital) visual cortex
PAIC	posterior agranular insular cortex
PAG	Periaqueductal gray
dPAG	dorsal Periaqueductal gray

vPAG	ventral Periaqueductal gray
PBN	(pontine) Parabrachial nucleus
pCREB	phosphorylated CREB
PBS	phosphate buffered saline
PDS	paroxysmal depolarizing shift
PDR	perceptual-defensive-recuperative
PHA-L	*Phaseolus vulgaris leukoagglutinin*
PKA	cyclic-adenosine monophosphate-dependent protein kinase
PKC	calcium phospholipids-dependent protein kinase
PL	prelimbic cortex
PLC	phospholipase C
PoT/PIL	posterior intralaminar nuclei of the thalamus
PIN	posterior intralaminar nucleus of the thalamus
POM	medial posterior complex of the thalamus
PPI	pre-pulse inhibition
PRh	perirhinal cortex
PRHC	parietal rhinal cortex
PTSD	Post-Traumatic Stress Disorder
PTN	posterior thalamic nucleus
PVA	parietal ventral area
Rp-cAMPS	Rp-adenosine 3 5'-cyclic monophosphothioate triethylamine
RPC	reticularis pontis caudalis

rVAF	rostral ventral amygdalofugal pathway
S1	primary somatosensory region
S2	secondary somatosensory region
SN	substantia nigra
SGN	supragenicular nucleus of the thalamus
STM	Short-Term-Memory
STP	Short-Term-Potentiation
Te1	primary temporal (auditory) cortex
Te2	secondary temporal (auditory) cortex
Te3	tertiary temporal (auditory) cortex
Te2D	secondary temporal (auditory) cortex dorsal portion
Te3R	tertiary temporal (auditory) cortex rostral portion
TM1 to TM7	Transmembrane spanning regions 1 to 7
TMT	trimethylthiazoline
TPOA	taste potentiated odour aversion
UCR	Unconditioned reflexive response
UCS	Unconditioned Stimulus
VAF	ventral amygdalofugal pathway
vdMGN	ventral division of the Medial Geniculate nucleus of the thalamus
VGCC	voltage-gated calcium channel
VGCCs	voltage-gated calcium channels
VDCC	voltage-dependent calcium channel
VDCCs	voltage-dependent calcium channels

VTA	ventral tegmental area
ZI	Zona Incerta

Abstract

The basolateral amygdala (BLA) contains NMDA, AMPA, and dopamine (DA) D_1 and D_2 receptors and neurobiochemical events within the amygdala mediate conditioned-fear-learning (CFL) and fear expression. Long-term potentiation (LTP) occurs in the amygdala during Pavlovian fear conditioning and is associated with fear-memory storage. CFL depends on NMDA, AMPA and dopaminergic receptor-mediated processes and enhanced amygdaloidal synaptic transmission facilitates fear-memory retrieval and makes the expression of conditioned fear possible. Since mesoamygdaloid DA receptors contribute to CFL and fear expression, Experiment 1A and 1D examined the impact of intra-BLA infusion of the DA D_1 and D_2 receptor antagonists SCH 23390 and raclopride L-tartrate on the acquisition of FPS in rats. Rats of the Wistar strain were bilaterally implanted with guide cannulae positioned 1.0 mm above the medial portion of the BLA. Approximately fourteen days later rats were assessed for baseline acoustic startle responding and assigned to drug-treatment groups. Forty-eight hours later rats were infused with either saline or the appropriate dopaminergic antagonist. The intra-BLA infusions occurred before five fear conditioning and testing blocks and were conducted to see if antagonism of DA receptors would prevent FPS acquisition. Retention testing for FPS took place forty-eight hours later. The results demonstrated that blockade of amygdaloid DA D_1 and D_2 receptors prevented the acquisition of FPS. The pretraining intra-BLA infusions of either raclopride or SCH 23390 disrupted the formation of long-term conditioned fear memories as rats treated with these DA antagonists failed to exhibit FPS on the retention test. Thus, the deficits in FPS displayed by SCH 23390 and raclopride-treated rats are likely due to the impact of these DA antagonists on associative learning and fear-memory consolidation processes. Experiment 2 demonstrated that fear-extinguished rats exposed to unsignalled footshocks displayed a reinstatement of FPS, but the exact neurobiochemical events involved in FPS reinstatement have not been elucidated. In contrast, fear-extinguished rats that received no unsignalled footshocks exhibited no FPS during final testing. Since unsignalled footshocks produced robust FPS reinstatement, Experiments 3A to 3D independently examined the effects of NMDA, AMPA, and DA D_2 and D_1 receptor antagonists on this phenomenon. Over a period of ten days, rats with cannulae targeting the BLA were baselined, fear-conditioned, pretested, fear-extinguished and then infused with either raclopride L-tartrate

(8.0μg, 4.0 μg and 2.0 μg), SCH 23390 (4.0 μg), (±)-2-amino-5-phosphonopentanoic acid {(AP5); 2.5 μg and 1.25 μg}, 6-Cyno-7-nitroquinoxaline-2,3-dione disodium {(CNQX); 5.0 μg and 2.5 μg), or phosphate buffered saline (PBS) before exposure to five unsignalled footshocks. FPS reinstatement was assessed 24 hours later. Results from these experiments demonstrate that PBS-infused rats showed FPS reinstatement, whereas rats infused with AP5, CNQX, or the two higher doses of raclopride failed to exhibit FPS reinstatement. Intra-BLA SCH 23390 infusions did not appear to disrupt the reinstatement of FPS in Experiment 3B, however obstructed guide cannulae may have affected these results. In Experiment 3C, intra-BLA AP5 infusions made just before unsignalled footshock presentation, prevented rats from exhibiting FPS reinstatement during final testing. A similar effect on FPS reinstatement was produced by CNQX application to the BLA of rats in Experiment 3D. The overall findings of Experiment 3 suggest that DA D_2 receptor antagonism and the glutamatergic receptor antagonists (AP5 and CNQX) impaired amygdaloid fear-memory reconsolidation and retrieval processes by preventing the re-excitation of neurons and pathways that had become established during fear-conditioning. It is speculated that these drugs may have interfered with excitatory synaptic transmission processes and neurobiological intracellular cascades within the amygdala and thus prevented FPS reinstatement from occurring. Two expression-control experiments (Experiments 4 and 5), revealed that the observed blockade of FPS reinstatement in Experiment 3 could not be attributed to the drugs simply blocking fear expression since infusion of raclopride, AP5, or CNQX into the BLA of non-extinguished rats 24 hours before final testing did not prevent rats from expressing FPS. Electrical stimulation (ES) of the human amygdala and temporal lobe region produces emotionally charged memory flash-backs and behaviours indicative of a central fear-state. ES of the rat amygdala is known to elevate acoustic startle amplitudes and enhance emotionality in rats and kindling of the rat amygdala exaggerates FPS and produces a variety of autonomic and behavioural fear responses. In rats, conditioned fear and LTP are induced by ES of amygdaloid afferents so it is possible that electrical excitation of BLA neurons can trigger FPS reinstatement. Experiments 7A and 7B were conducted to test the hypothesis that ES of BLA neurons can restore FPS responding in fear-extinguished rats. Thus, rats with bipolar electrodes implanted unilaterally in the BLA were baselined, fear conditioned, pretested

and then assigned to one of five groups matched on FPS. Rats then received fear-extinction or no-extinction training, followed 48 hours later by either 100 unsignalled electrical stimulations of the BLA or no stimulation. Experimental groups included; [Extinction + Stimulation (N=12), Extinction + No stimulation (N=12), No Extinction + No stimulation (N=12), No Extinction + Stimulation (N=11) and Extinction + Stimulation out of context (N=12)]. FPS reinstatement was assessed 24 hours later and BLA AD-current threshold were recorded four days after FPS reinstatement testing was completed. Experiment 7A demonstrated that all experimental groups exhibited FPS except the Extinction + No stimulation control group which displayed a robust extinction effect. Most importantly, the Extinction + Stimulation group exhibited statistically significant FPS reinstatement after ES of the amygdala. Experiment 7B demonstrated that ES of the amygdala in a context different to the startle apparatus also produced FPS reinstatement in fear-extinguished rats. However, the magnitude of this FPS reinstatement effect was much smaller than that obtained when rats received ES of the amygdala in the startle testing apparatus. The overall finding that ES of the BLA causes FPS reinstatement in fear-extinguished rats suggests that ES of the BLA resensitised fear-memory systems and restored FPS responding. This effect was likely achieved by exciting amygdaloid neurons and pathways containing the memory-trace of the CS-UCS association originally established during Pavlovian fear conditioning. Experiments 8 to 10 used the same protocol as Experiment 7A and investigated whether ES of other brain regions that share reciprocal connections with the amygdala would trigger FPS reinstatement in fear-extinguished rats. Rats in Experiments 8 to 10 had bipolar electrodes unilaterally implanted in the perirhinal/insular cortex (PRh/IC), dorsal periaqueductal gray (dPAG), ventral tegmental area (VTA) and ventral periaqueductal gray (vPAG). Experiment 8 revealed that ES of the PRh/IC produced a positive trend towards FPS reinstatement. Experiment 9 examined the impact of dPAG and VTA ES on FPS reinstatement. This experiment demonstrated that VTA but not dPAG ES produced a FPS reinstatement effect. This finding seems to support the research evidence that highlights the importance of mesoamygdaloid systems in mediating conditioned fear and stress responses. Experiment 10 assessed the impact of vPAG ES on FPS reinstatement and discovered that stimulation of this region did not trigger a restoration of FPS in fear-extinguished rats but it did seem to elevate overall acoustic startle

responding during final testing. This finding would seem to indicate that ES of the vPAG likely increased contextual fear but not cue specific fear since acoustic startle amplitudes during both the noise-alone and the CS + noise conditions were elevated. The key finding that ES of the amygdala produces robust FPS reinstatement provides further proof that the amygdala and its afferent and efferent neural circuits are essential for fear-memory reconsolidation and conditioned fear expression and reinstatement.

Introduction

Today, as in the past, emotions continue to fascinate philosophers, scientists and society in general. Great literary works, political movements, movies, and individual behaviour have all been influenced by fear, joy, love, anger, frustration and sorrow. In this way fear and other emotions add a vibrant colour to our world and to our individual and most intimate thought processes. Indeed, fear and various other well defined emotions have infused themselves into the behavioural repertoire of each individual and into the much larger fabric of society. Highly charged emotions such as fear motivate not only the way we think but also how we respond to sensory stimuli in our environment. More important however, is the idea that fear is biologically adaptive, in the sense that it sets off a cascade of autonomic and behavioural responses that are designed to help us avoid or escape from dangerous situations. Despite this obvious benefit, fear and the anticipatory anxiety that follows can also be detrimental, as when combined, they can act like a dual edged sword. On the one hand, fear and anxiety can protect an individual from danger, but on the other, in their extreme form, they can cause emotional problems and severe disorders of thought or mood that can produce maladaptive behaviours that place the afflicted individual in peril.

Undoubtedly, one of the most powerful negative emotions to have captivated humanity's interest is fear. Over several centuries, the study of fear and emotionality has progressively migrated out of the spiritual realm and the field of philosophy, and is now firmly established in the field of physiological psychology which examines how fear learning and expression occurs at the neuronal and molecular levels in the brain (LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998; LeDoux, 2002). In the past, and before the scientific method of studying emotionality was established, most of the underlying mechanisms contributing to fear learning and emotional behaviour were part of the great unsolved mysteries of the mind. At around the end of the nineteenth and the early part of the twentieth century things began to change as far as the study of fear and emotionality was concerned. During this period many neurological theories of fear behaviour and emotionality were developed as were the numerous animal models of fear learning and expression that were employed to unravel the neural mechanisms underlying fear (LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998). These early scientific efforts contributed

significantly to our present day understanding of the brain systems that mediate fear behaviours and emotional learning. Today, the field of neurosciences is still working diligently at delineating the neural mechanisms and biochemistry of fear and anxiety and over the past few years some significant progress has been made, for much of what was once considered mysterious or outside the bounds of human comprehension can now be explained with a greater degree of certainty. Considerable advancements in laboratory conditions, computerization and recording equipment, cellular and molecular biochemistry, genetics, and behavioural testing equipment and methods have all made the study of fear and emotionality more precise. However, despite these scientific advancements, some mysteries concerned with how fear is learned, consolidated and stored in the brain still remain.

While the primary focus of this Ph.D. is on emotionality and the neurobiological and electrophysiological mechanisms of fear learning and fear reinstatement, it can also be described as a journey, whose goal is to take the behavioural correlates associated with fear and redefine them in terms of neurobiological and synaptic processes that occur in discrete areas of the brain. Since it is often important to place current work into a historical context, this journey will begin in Chapter One with an examination of how the philosophical inquiry into fear and emotionality progressed towards more empirically based models of investigation. Chapter Two examines how early physiological and neuroanatomical advancements and efforts evolved into a newly emerging field of physiological psychology which began to study how emotions such as fear are learned, experienced and expressed in the brain. This particular chapter will provide a historical overview by discussing some of the early theoretical views on fear and emotionality and by summarizing the ideas and research findings of many great physiologists and psychologists. Particular emphasis will be placed on highlighting the most important aspects of each researcher's position regarding how emotions are learned, expressed and subjectively experienced. Within the context of each of these descriptions the neuroanatomical substrate that was believed to mediate emotional behaviour will be discussed. Chapter Two will culminate with the limbic theory of emotion and will highlight its shortcomings and positive lasting legacies while also introducing the historical evidence implicating the amygdala as a key brain region that is involved in the acquisition and expression of conditioned fear.

Chapter Three will move into a discussion of the anatomy and connectivity of the amygdala with some mention of the various receptor subtypes that are located in this limbic structure. This chapter will place particular emphasis on the cortical and subcortical projections to the amygdala and on amygdaloid efferents to cortical and various midbrain nuclei. The contents of Chapter Three will also describe the intrinsic connections between various amygdaloid nuclei and how they may be related to fear learning and expression. Thus, Chapter Three provides the reader with some of the background to the neuroanatomical and neurochemical properties of the amygdala. This is important as some of the terms used in Chapter Three will inevitably come up in later chapters and the general discussion that follows the experimental results.

Chapter Four focuses on the role of the amygdala in emotion, fear and unconditioned fear effects. This chapter first discusses the amygdala's participation in unconditioned fear responding, innate fear expression, stress and anxiety-like behaviour. Within this context some theoretical discussions are introduced in order to make distinctions between unconditioned fear and conditioned fear effects and how an internal fear state develops in response to aversive stimuli. Next, Chapter Four explores how amygdaloid dysfunctions are related to inappropriate emotionality especially as it pertains to deficits in fear expression. These discussions will demonstrate how emotional and behavioural disruptions exhibited by primates and other mammalian species with amygdala damage are similar to the emotional pathology observed in those who suffer from severe schizophrenia. Particular emphasis will be put on how the amygdala and an over active mesocorticolimbic dopamine system may be the underlying causal agents involved in this mental disorder.

Following these discussions, Chapter Four will shift focus and demonstrate how excitation of amygdala neurons influences fear expression and stress responding. Some emphasis will be placed on how the amygdala-bed nucleus of the stria terminalis fear and anxiety circuit mediates unconditioned fear and anxiety-like responding. Chapter Four will conclude by examining how amygdaloid and septal area interactions facilitate unconditioned fear responses and how excitation of the human amygdala is linked to fear, anxiety, and fear-provoking memories.

Chapter Five will present an extensive literature review highlighting the amygdala's role in fear acquisition and expression. Chapter Five will begin with a brief and general

overview of the role of the amygdala in fear learning and expression and then will move on to examine the amygdala's involvement in mediating conditioned fear responses. This chapter will then review the various lesion studies carried out in the amygdala that point to its involvement in fear acquisition and expression. Chapter 5 will culminate in discussions that deal with the possibility that many conditioned fear memories may be stored within the amygdala. In doing so, Chapter 5 will introduce the reader to the next topic which deals with the cellular, biochemical and synaptic mechanisms thought to be involved in fear-learning and fear memory consolidation. This new topic will be covered in Chapter 6.

Chapter 6 will provide a detailed account of long-term-potentiation (LTP). This chapter will first provide a theoretical overview of how LTP is linked with Pavlovian conditioning, CS-UCS learning and memory formation. After this Chapter 6 will provide a description of the biochemical cascades associated with LTP and how these may be related to Pavlovian fear conditioning. These discussions will describe how calcium entry into the postsynaptic cell sets off a chain of events that activates several protein kinase signalling pathways believed to be responsible for instigating synaptic modifications and learning. Research findings from several different Pavlovian fear conditioning paradigms will be incorporated into these discussions in order to supplement this section. After these discussions are complete, attention will shift to the involvement of amygdaloid glutamatergic receptors (i.e. N-methyl-D-Aspartate [NMDA], Alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionoic acid [AMPA], metabotropic glutamate receptors [mGluRs]), in conditioned fear learning and expression. Some discussions dealing with the role of benzodiazepine and gamma-aminobutyric acid (GABA) in conditioned fear learning and expression will also be included. The involvement of dopamine systems and dopamine amygdaloid receptors in mediating the acquisition and expression of fear will be reviewed near the end of Chapter 6 and this section will be given special attention since many of the experiments contained within this thesis use drugs that antagonize dopamine receptors in the amygdala and central nervous system (CNS). The emphasis of this particular section will be on the mesolimbic and mesocorticolimbic dopamine systems and their contribution to fear learning, expression and emotionality. There will also be a brief explanation of the fear-potentiated startle paradigm that outlines its evolution and lists some of its advantages.

Finally, Chapter 6 will culminate with a thesis statement outlining the purpose of the Ph.D. The methods and results section follows Chapter six and all this experimental work can be found in Volume 2 of this thesis. After each major experiment a brief discussion section will follow in order to provide an explanation of the results. At the end of the experimental section a general discussion (see Volume 3) will link together the present research and will provide an overall explanation of the results as they relate to the rest of the research literature on fear learning and expression. Implications for future research and an overall conclusion will complete the thesis.

Chapter 1

History of Emotion: From Philosophical Inquiry to Scientific Investigation

1.1: The study of Emotion: Philosophical Beginnings

Physiological psychology's modern day study of emotion had its early beginnings in the discipline of philosophy and humanity's interest in emotions has been with us since recorded history began. Indeed, some of the earliest writings regarding human nature and human emotionality were undertaken by the philosophers. Perhaps the earliest attempt to ascribe emotionally charged behaviour to events occurring in the brain was made by Hippocrates (460-377 $_{B.C.}$) in the fifth century B.C. Other philosophers who followed such as Jean Jacques Rousseau (1712-1778), Thomas Hobbes (1588-1679), and René Descartes (1596-1650) attempted to explain how emotions, both positive and negative served to motivate human behaviour and affect social or political relationships. In essence, philosophy at this time was trying to grapple with explanations of human nature and emotion in order to understand how humans fit into the cosmos. As a result, these philosophers did not aggressively study the underlying mechanisms that contributed to emotional experiences and behaviours per se' but rather used emotions as a way to help define human nature and motivation for the purposes of establishing a stable society. Despite this fact, it is important to recognize that these early philosophers did not take emotion for granted, rather they believed that emotion and the ability to reason, learn, or solve complex problems was what made humans unique and separated the human species from the animal kingdom (for a review on Rousseau see Grimsley, 1973; for a review on Descartes see Clarke, 2003; for a review on Hobbes see Malcolm, 2002).

Jean Jacques Rousseau (1712-1778) recognized that humans tend to oscillate between what he called intimacy and isolation or the appetitive and aversive motivational states. Rousseau suggested that all humans are intrinsically good and tend to approach stimuli which are rewarding and thus seek intimacy and the positive emotional states that follow. Rousseau referred to this behaviour as following the passionate instincts of the heart over the dictates of reason. Although Rousseau advocated positive instinctual and passionate emotional feelings over intellectual reasoning he still recognized that humans use their intellectual abilities to avoid aversive events and to cope with stressful situations.

Rousseau felt that repeated and prolonged exposure to negative stimuli or events could exhaust the human capacity to cope and thus cause people to seek isolation (see Rousseau 1712-1778 in Gleitman, 1981; Grimsley, 1973).

Thomas Hobbes (1588-1659) adopted a radically different approach to human nature and emotionality by suggesting that humans are much more calculating and driven by negative emotional states. According to Hobbes humans are continuously adding up the positive and negative aspects of a situation in an internal mathematical equation that eventually motivates them to action. Unlike Rousseau, Hobbes argued that human nature is dark and destructive and human behaviour is to a large extent driven by selfish motives and an overpowering fearfulness of a sudden violent death (Malcolm, 2002; Gleitman, 1981).

Perhaps the most important contribution made by Hobbes concerns the strong emphasis he placed on fear as an emotion that motivates humans into action. Indeed, Hobbes often referred to fear as his twin and life long companion. In essence, Hobbes was firmly aware that negative emotions associated with the fear of a quick and horrible death are what lead individuals to enter social contracts designed to protect life, liberty, and estate. Without fear and social arrangements to govern human behaviour, life, according to Hobbes would be brutish, nasty and short. Thus, Thomas Hobbes viewed the emotion of fear associated with threatening stimuli (real or imagined) as a very powerful internal motivational state that fulfilled the self preservation function for the human species (Malcolm, 2002; also see Hobbes 1588-1659 in Gleitman, 1981).

1.2: René Descartes: The Mind-body Dichotomy and the Simple Reflex Arc

At around the same time that some branches of philosophy were grappling human emotion as it related to human nature, the first real efforts to understand the neurobiological mechanisms underlying fear and emotionality began with the work of Descartes (1596-1650). Although Descartes was a philosopher by trade, he still recognized that most behavioural responses are the by-product of external events or stimuli interacting with our internal nervous system. Descartes observed that all actions or behaviours, whether initiated by humans or animals, were essentially a response to some event or stimuli in the outside world. Descartes suggested that some event or stimulus excites one of the senses (touch, vision, hearing or some other bodily sensation) in our peripheral system and this

then excites a nerve which transmits the excitation upward towards the brain via animal spirits. Information from the brain is then sent back to the muscles and organs, causing contractions of the muscle and various changes in bodily organs in reaction to the external event (Clarke, 2003; also see Descartes in Gleitman, 1981; see Descartes in LeDoux and Hirst, 1986 and in LeDoux, 1998).

Descartes envisioned the senses operating in conjunction with a series of hollow tubes used for passing information to the brain from sensory systems or organs. It is important to note, however, that Descartes made a clear distinction between the brain which belonged to the body and the mind which belonged to the non-physical and spiritual entity called the soul that was housed in the pineal gland. Thus, according to Descartes, sensory information requiring logical reasoning was processed in the brain by the pineal gland that housed the soul. In contrast, most automatic or reflexive responses such as respiration, circulation of bodily fluids and flight from danger were controlled by the brain without any intervention from the mind. In this way Descartes recognized that some of our actions and bodily functions are automatic or reflex-like and are therefore controlled by mechanical principles and not by human will, whilst other behaviours involving emotions and intellectual capacities are controlled by the mind. This view became known as the mind-body dichotomy and even today it greatly influences academic writing and scientific inquiry (Clarke, 2003; also see Descartes, 1596-1650 in Gleitman, 1981; see Descartes 1596-1650 in LeDoux and Hirst, 1986 and in LeDoux, 1998).

Perhaps, Descartes' most important contribution to future inquiry was his recognition that a simple reflexive response could be directed in response to a stimulus even without conscious effort and that conscious awareness of emotions mediated by the mind could influence behaviour. In doing so, Descartes provided philosophical and scientific inquiry with the first explanation of the reflex arc and a simplified neurological model of emotion that linked the machinations of the mind to the brain and various bodily functions. Those who followed Descartes, such as Julian Offray de La Mettrie (1709-1751) began to challenge philosophical and religious doctrines which had seen human behaviour as directed by a soul that was in contact with the spiritual world. La Mettrie extended Descartes views by arguing that human beings were not all that different from animals. La Mettrie contended that the only observable difference between the animal and humans was

that humans were more intricately constructed and far more complex. Generally speaking, La Mettrie's considered human beings as complex reflex-machines, and any behavioural or emotional responses that occurred, were to a large degree, determined by events in the environment (Clarke, 2003; see Descartes 1596-1650 in Gleitman, 1981; also see Descartes in LeDoux and Hirst, 1986 and in LeDoux, 1998; see Julian Offray de La Mettrie 1709-1751 in Gleitman, 1981).

These early philosophical ideas on emotion and reflexes generated interest within the scientific community to begin studying human physiology and emotion more empirically. In fact, some of the psychological terms used today have been passed down from early philosophical and scientific inquiry. For example, psychology uses the appetitive and aversive motivational labels to describe animal and human behaviour that is directed towards a specific cue or stimulus that predicts a rewarding or aversive event. Moreover, physiological psychology still recognizes the importance of measuring reflexive responses and how they are linked to appetitive and aversive motivational states and how some of these states are represented and produced by the central nervous system working in conjunction with the peripheral nervous system. Finally, psychology recognizes that a powerful emotion such as fear and the behaviours associated with this emotional state can be used to study the intricate neurobiological and neurophysiological processes underlying its genesis.

Despite some of the contributions made by many of these early philosophers, the study of emotions and the reflexive or behavioural responses associated with them remained a philosophical inquest until the mid to late nineteenth century. This was likely due to the prevailing belief that emotions were subjective and internal states and that any behaviors or reflexes arising from them was controlled exclusively by the soul or spiritual forces that were inaccessible to direct scientific investigation. Thus, for nearly two hundred years after Descartes death, physiologists and neuroanatomists could not come up with a parsimonious explanation of how even a simple reflex was transmitted in the human body, let alone even consider how emotional states could modify such reflexes or even how reflexes could be modified through learning and memory processes that involved the brain.

Chapter 2

A History of Emotion and Fear: From Physiology to Physiological Psychology

It was not until the mid-nineteenth and early twentieth century that the study of nerve impulses, emotions, and learning was made accessible to empirical investigation. Emil DuBios-Reymond (1848, 1849) using a galvanometer and faradic stimulation techniques was the first researcher to demonstrate the negative current variations that take place in a nerve during active periods. DuBios-Reymond's (1848, 1849 in Sheer, 1961) studies helped formulate the basis for the theory of polarization and propagation of the nerve impulse and demonstrated that the transmission of nerve impulses could be explained by the electrical and chemical properties of particles arranged along the surface of a nerve (Sheer, 1961). A decade later, the physiologist Hermann von Helmholtz (1850) was the first to demonstrate that the velocity of a nerve impulse could be measured by stimulating the nerve and then measuring the time taken to produce a reflexive response (von Helmholtz, 1850 in Sheer, 1961; also see Gleitman, 1981). The combined work of Herman von Helmholtz and Emil DuBois-Reymond effectively removed the study of nervous activity out of the mystic realm of animal spirits and placed it firmly into the experimental laboratory. These types of scientific advancements in measuring devices made it possible for several prominent physiologists and neuroanatomists (e.g. Bell, 1826; Muller 1801-1858; Munk, 1890; Ferrier, 1886; Fritsch and Hitzig, 1870) to not only map the path taken by sensory and motor nerves as they travelled in the periphery to innervate visceral and skeletal target areas but also to demonstrate that a variety of sensory and motor functions performed by limbs and external sense organs were represented in different parts of the cortex (for a review see Sheer, 1961; LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998). This paved the way for researchers to construct theories and conduct research into how emotionally charged reflexive responses and learned associations are formed and represented in the brain and how these three processes could combine to influence behaviour.

The first serious attempt to link reflexive responses and learning was made by Sechenov (1829-1905; 1863) who suggested that certain types of mental activity can become linked to bodily responses and reflexes. According to Sechenov (1863) certain tasks such as

reading and writing were clear examples of reflexive modifications in that they involved the forming and strengthening of associations between cortical areas responsible for initiating mental processes with those brain regions responsible for coordinating physiological responses. Sechenov (1863) even proposed that unrelated mental events can become associated if are repeatedly paired with each other, and eventually one event will elicit the other. Sechenov (1863) adopted the view that a similar linked association could be developed and maintained between reflexive responses, thus allowing one reflexive response to modify another one. Moreover, Sechenov (1863) recognized that neutral external stimuli have the capacity to become associated with reflexive responses to the point where exposure to the external stimuli alone is sufficient to trigger the desired reflexive response. In essence, Sechenov suggested that external stimuli leave a memory-trace in the brain and that this trace can be evoked by the reappearance of any part of the original stimulus that had been paired with the reflex being examined. Thus, Sechenov's contribution was his view that reflexive responses as well as the psychological phenomena occurring during learning and memory formation could be scientifically studied for the purposes of establishing precisely where in the brain certain memories reside (for reviews on Sechenov's work see Koshtoyants, 1964; 1965; Sheer, 1961; Subkov, 1935).

Sechenov's ideas were to a large degree validated by the seminal experimental work carried out by Sherrington (1900; 1906) and Pavlov (1927). Sherrington's (1900; 1906) research demonstrated that more complex patterns of behaviour could be formed by chaining together simple reflexes and that visceral feedback to the brain did not contribute as much to emotionality as was earlier predicted by the James-Lange (1890) theory of emotion. Sherrington (1906) also demonstrated how the brain and central nervous system (CNS) integrates and summates synaptic signals. Sherrington (1906) accomplished this task by investigating how nerve impulses are spatially and temporally sequenced in certain neural pathways that elicit reflexive responses. From this research Sherrington (1906) was able to deduce that excitatory and inhibitory signals are represented in neurons at the level of synapses. The functional task of the nervous system was to summate these signals to cause excitatory or inhibitory reflexive responses to emerge out of the plethora of neurosynaptic events. Sherrington (1906) referred to the spatial and temporal summation of synaptic input processes occurring at the cellular level as the functional integration and

action of the nervous system. This model simply states that all neurons must make an all or none decision, that is to either fire an action potential causing excitation or to suppress the action and induce inhibition (see Sherrington in Gleitman, 1981; Kolb and Whishaw, 1990; LeDoux, and Hirst, 1986; Kandel and Seigelbaum, 2000).

Generally speaking, Sherrington's (1900; 1906) and Ivan Pavlov's (1927) ground breaking work set the stage for the development of theories and research into synaptic transmission and cellular models of learning and memory. Researchers in this field proposed that enhancements in synaptic efficiency could emerge out of spatial and temporal stimulus presentation patterns that occur during Pavlovian classical conditioning training procedures (see Pavlov, 1927; Konorski, 1948; Hebb, 1949). Two of the key proponents of cellular models of learning and memory were Jerzy Konorski (1948) and the distinguished Canadian psychologist Donald Hebb (1949). Basically, Hebb (1949) suggested that if two proximally situated neurons in a pathway (one presynaptic and the other postsynaptic) are activated at the same time then the degree of connectivity between them will be significantly strengthened (Hebb, 1949; also see Hebb, 1949 and Konorski, 1948 in LeDoux, 1998; 2002). This model of cellular memory and synaptic efficacy is often called the wiring by firing theory and essentially states that "neurons that fire together wire together" (see Shatz, 1999 in LeDoux, 2002). Konorski (1948) proposed ideas that were very similar to those of Hebb (1949) except Konorski employed the term "plasticity" to provide a descriptive account of how neurons can become transformed by Pavlovian conditioning procedures and experience. However, it must be emphasised that none of these models would have been possible had it not been for efforts made by Ivan Pavlov (1927).

Pavlov's (1927) influential work on classical conditioning added to Sherrington's (1900; 1906) research and significantly expanded our knowledge of the physiological, biological, and stimulus factors which influence learning and memory. In general, Pavlov's (1927) classical conditioning experiments illustrated that if a neutral stimulus (i.e. a bell or a light) is repeatedly paired with a cue or unconditioned stimulus (UCS; e.g. food) that produces an unconditioned reflexive response (UCR; e.g. salivation) the neutral stimulus through a learned association becomes a conditioned stimulus (CS) that is capable of eliciting the reflexive response (e.g. salivation) in the absence of any food. Pavlov (1927) suggested

that a learned association or a stimulus-stimulus connection develops between the CS and UCS after repeated pairings.

Pavlov (1927) hypothesized that following classical conditioning (CS-UCS pairings) the CS activates areas of the cerebral cortex that are normally aroused by the UCS, and this in turn arouses efferent control centres in the brain responsible for producing the UCR. Pavlov (1927) and various other researchers who followed (Prosser and Hunter, 1936 Konorski, 1948; Hebb, 1946; Hebb, 1949; Brown, Kalish and Farber, 1951; Davis, 1992a; 2000; LeDoux, 1992; 1998; 2000; 2002; Fendt and Fanselow, 1999; Maren, 1999) found that many of the principles of classical conditioning and chaining of reflexes demonstrated by Sherrington (1906) could also be applied to fear learning and fear expression. For example, more aversive secondary unconditioned stimuli (i.e. shock or heat) could be used to enhance or sensitise reflexive responses to a primary UCS (i.e. the acoustic startle reflex or withdrawal reflex) as long as the aversive secondary UCS preceded the primary UCS or if they shared a close temporal relationship. This temporal relationship is also referred to as the contiguity principle and basically states that conditioned responses will be acquired more rapidly when the CS precedes and is paired with the secondary aversive UCS than if the CS is unpaired (Pavlov, 1927; Konorski, 1948). Prosser and Hunter (1936) used reflexive-chaining experiments to study the extinction of startle responses and spinal reflexes in laboratory rats and found that shock presentation could significantly increase acoustic startle response magnitudes. Based on this work, Prosser and Hunter (1936) and various other researchers (Kemp and Coppeé, 1936; Lorente de Nó; 1933; Culler and Mettler, 1934) were able to apply reflexive chaining and classical conditioning techniques to examine startle response latencies that helped map the acoustic startle pathway from the cochlea to the inferior colliculus. The chaining of events and reflexes as well as the temporal order of stimulus presentation is a prominent feature found in most classical conditioning paradigms that examine fear learning today (for review see Davis, 1992; 2000; LeDoux, 1992; 1998; 2000; 2002; Fendt and Fanselow, 1999). Thus, in a typical Pavlovian fear-learning paradigm, such as the fear-potentiated startle (FPS) paradigm (see Brown, Kalish and Farber, 1951; Davis, 1992a; Davis, 2000; LeDoux, 1992; 2002), a neutral stimulus (i.e. light or tone) that is repeatedly and temporally paired with a more aversive secondary UCS (i.e. footshock), can, through associative learning processes become a CS

capable of evoking a conditioned reflexive response that is significantly greater in magnitude than the unconditioned reflexive response (UCR) normally produced by the primary UCS (i.e. white noise burst). The difference between the CR that is elicited in the presence of the CS (i.e. light) and the UCR (i.e. noise alone startle) which is elicited in absence of the CS, is considered to be the magnitude of conditioned fear or the fear effect (see Davis, 1992a,b).

It is important to point out that elevated acoustic startle responding in the presence of a stimulus (i.e. light or tone) previously paired with an aversive footshock (UCS) is considered to be a fear-induced response that occurs as a result of learning. In other words, if the once neutral stimulus (i.e. light or tone) is repeatedly paired with an aversive footshock it becomes a CS that predicts danger. When this CS is presented just prior to the onset of a white-noise burst, the measured acoustic reflexive response amplitude will be much higher than if no CS is administered before the white-noise burst. Hence, fear conditioned animals will typically exhibit much higher acoustic startle amplitudes on CS + noise trials than on noise-alone trials (Brown, et al., 1951; see Davis, 1992a,c; 2000).

Another important legacy Pavlov (1927) left the neurobehavioural sciences was the fact that excitatory conditioned responses could be inhibited or extinguished by repeatedly presenting the CS alone. According to Pavlov (1927) and many other prominent researchers (Prosser and Hunter, 1936; Konorski, 1948; Hebb, 1946; Hebb, 1949; Rescorla-Wagner, 1972; Rescorla and Heth, 1975; Bouton, 1980; 1983; Davis, 1992; Walker and Davis, 2002; LeDoux, 1998; 2002; Westbrook, 2000) this phenomenon called "extinction" really represents a form of learning called inhibitory learning. Pavlov (1927) and those who followed up on his work (Prosser and Hunter, 1936; Konorski, 1948; Hebb, 1946; 1949; Rescorla and Wagner, 1972; Rescorla, 1979; Boutin, 1993) viewed extinction as a form of learning that was able to exert control over, mask, or inhibit the excitatory memories and neurosynaptic processes that had been established during the original CS-UCS training regimé (e.g. light + footshock pairings). In terms of fear learning, it is important to note that extinction does not imply forgetting, since extinguished animals often show a robust reinstatement of conditioned responding to a CS following re-exposure to the UCS (i.e. footshock) (Rescorla and Wagner, 1972; Rescorla and Heth, 1975; Rescorla, 1979; Heth, 1975; Bouton, 1980; 1983; Bouton and Bolles, 1979; Walker and

Davis, 1997; Gewirtz, Falls, and Davis, 1997; Westbrook, Iordanova, McNally, Richardson and Harris, 2002). The reinstatement of fear caused by re-exposure to the UCS highlights the fact that fear conditioning and extinction training are represented in the brain as distinct CS-UCS and CS-no-UCS memories and fear reinstatement responding to the CS depends on which memory is activated (Bouton and Bolles, 1979; Bouton, 1993). This reinstatement effect has been demonstrated across a number of laboratories that use classical fear conditioning techniques to study the neurobiological process of fear-learning and memory (Bouton and Bolles, 1979; Walker and Davis, 1997; Gewirtz, et al., 1997; Walker and Davis, 2002).

Essentially, Pavlov's (1927) classical conditioning paradigm provided science with the foundation and mechanism to unravel how learned associations are formed at the neurobiological level. Indeed, Pavlov (1927) and various behavioural scientists who followed (Konorski, 1948; Hebb, 1946; 1949; Brown, et al., 1951) helped to establish the behavioural and theoretical framework currently used by the neuroscientific research community that is dedicated to the study of emotion and fear learning. In fact many cellular models of learning and memory grew out of this early work (see Hebb, 1949; Konorski, 1948; Lømo and Bliss, 1973; LeDoux, 2002) and these ideas served as the driving force behind today's current learning and memory models that examine the neurobiology of conditioned fear. An especially prominent present day example is long-term-potentiation (LTP) which is a biochemical and molecular based model of learning and memory that attributes changes and enhancements in synaptic efficiency that occur during Pavlovian fear conditioning to a form of cellular memory. It is hypothesized that this form of cellular memory is indicative of learning and thus is able to influence autonomic and behavioural responses that are typically associated with emotionality and a central fear state (for a review on LTP see Hebb, 1949; Bliss and Lømo, 1973; Bliss and Collingridge, 1993; LeDoux, 2002; Schafe, Nader, Blair, and LeDoux, 2001). A more detailed explanation of LTP will be provided in subsequent chapters (see Chapter 6).

2.1: Just what is an Emotion?

From the James-Lange Theory of Emotion to the Cannon-Bard Theory of Emotion

While some physiologists were busy attempting to unravel the physiological mechanisms and stimulus factors concerned with reflexive responses associated with speech and bodily movements (e.g. Broca, 1824-1880; Wernicke, 1848-1904; Ferrier, 1843-1928; 1886; Jackson, 1835-1911; Munk, 1890; in LeDoux, 1986 in LeDoux and Hirst, 1986; also see LeDoux, 1998) other researchers focused more efforts on understanding how emotions are produced in the brain and how these emotions are subjectively experienced (James, 1884; James-Lange, 1890 in LeDoux, 1986 in LeDoux and Hirst, 1986; also see LeDoux, 1998; Cannon-Bard, 1927; 1928; 1931). In terms of emotion, the James-Lange (1890) and Cannon-Bard (1927; 1928; 1931) theories were the first to provide science with a physiological explanation of how the subjective experience of emotions and the behaviours associated with them are produced. The James-Lange (1890) theory of emotion placed a great deal of emphasis on the role of visceral responses in the generation of subjective emotional states. This theory put forward the notion that emotional situations or events elicit specific sets of autonomic and visceral reactions. These reactions are received by the central nervous system which interprets them and then chooses the appropriate set of emotional responses.

William James (1884), a physiologist by trade, was highly impressed with the discoveries made in elucidating some of the sensory and motor centres in the cerebral cortex, by such prominent researchers as Jackson (1835-1911), Fritsch and Hitzig (1870), Ferrier, (1886) and Munk (1890). However, James (1884; 1890) felt that emotion and all the intrusive pains, pleasures, and fears of the mind had their own special centres in the cerebral cortex and that this important feature had been largely ignored by the research community. According to James' (1884; 1890) view, stimuli received by the sensory areas of the cortex have the capacity to excite motor areas and through descending pathways produce various visceral and skeletal responses. Information concerning changes in visceral and skeletal responses is sent back to special areas in the sensory cortex and the specific emotion is then experienced. To some degree, James (1884; 1890) was trying to establish the idea of emotional localization by suggesting that some areas of the cortex were devoted to mediating specific human emotions just as certain sensory and motor

functions associated with speech were observed to be localized (for a reviews on James, 1884; 1890 see LeDoux, 1986 in LeDoux and Hirst, 1986; also see LeDoux, 1998).

Many of William James' (1884) theoretical concepts and ideas about how emotion was experienced and constructed received a great deal of support from the work of Carl Lange (1885). Carl Lange's (1885) views concerning how the subjective experience of emotions are fashioned is actually very similar to that of William James (1884; 1890), with the exception that Lange (1885) focused more on cardiovascular changes as being the main bodily disturbance that produced an emotional state. Over the years these two views were combined to form the James-Lange (1890) theory of emotion. In the James-Lange (1890) theory the causal relationship appears to be reversed, that is the visceral response comes first and then the information is sent to the sensory centres in the cerebral cortex responsible for ascribing an emotion to fit the particular visceral response. In other words, humans feel afraid because they tremble and their heart races, or angry because they strike out, rather than the other way around. Thus according to the James-Lange (1890) theory of emotion, the feedback produced by visceral responses impinging on the sensory cortex is what caused the emotional experience (for a review on Lange, 1885 and the James-Lange theory of emotion 1890 see LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998). As neuroscientific methods concerned with the study of emotion became more rigorous and empirically based and as more information about brain organization was made available, the ideas underpinning the James-Lange (1890) theory of emotion began to be challenged by various researchers who provided evidence that subcortical brain areas may be responsible for mediating emotionality (Sherrington, 1900; Head and Holmes, 1911; Head, 1920; Dana, 1921; Cannon; 1927; 1931; Cannon and Bard 1928; 1931), the most prominent of these were Cannon (1927; 1931) and Bard (1928).

2.2: A Challenge to the James-Lange Theory of Emotion and the Role of the Thalamus in Emotion

The Cannon (1931) and Bard (1928) theory provided a more neurobiological and neuroanatomically based explanation of emotion and behaviour that was backed up by extensive laboratory experimentation. Based on their work, Cannon (1927; 1931) and Bard (1928) disagreed with the James-Lange theory and argued that visceral changes alone are

not the basis of emotion since stimulation of visceral organs or lesioning of autonomic afferents to the cerebral cortex does not alter emotional behaviour and since the artificial induction of visceral changes produced by adrenalin injection does not produce strong emotions. Furthermore, Cannon (1927) pointed out that sympathetic reactions to arousing stimuli are very similar while the reported emotional experiences may vary widely. For example, fear and rage produce a similar pattern of violent sympathetic discharge however the subjective emotions and behavioural patterns which accompany the two are qualitatively different. Cannon (1927; 1931) and Bard (1928) suggested that emotions such as fear and rage and their associated behaviours are mediated by a complex interaction between numerous cortical and sub-cortical structures working in conjunction with the sympathetic and parasympathetic nervous system processes. It is the interaction between the central and autonomic nervous system along with the release of adrenalin (epinephrine) that mobilizes an organism for flight or fight responses. Cannon (1929; 1931) argued that the viscera are relatively insensitive structures and visceral changes are too slow to be a source of emotional feeling as described by the James-Lange (1890) theory of emotion. Cannon (1929; 1931) based his argument on the fact that some visceral responses can take several seconds to several minutes to occur, whereas affective reactions to pictures or emotionally arousing stimuli occurred in less than one second. Thus, Cannon (1929; 1931) felt that visceral changes and a backflow of information from the peripheral nervous system to the brain could not adequately account for the entire range of human emotional experiences.

From a neuroanatomical point of view Cannon (1927; 1929; 1931) and Bard (1928) believed that emotions are generated by the thalamus which transmits and receives enormous quantities of somatosensory and visceral information from various cortical, sub-cortical, and brainstem structures. As such, the thalamus was believed to participate in the control and regulation of the somatic, behavioural, and visceral aspects of emotional arousal whether it had a positive or negative valence. The Cannon-Bard (1928; 1931) theory proposes that all information concerning sensory stimuli gains access to cortical and sub-cortical structures via the various thalamic nuclei, and any stimuli that induce radical emotional change are independently processed within the thalamus. According to this view, the processed sensory information in the thalamus is simultaneously transmitted to

the cerebral cortices resulting in the cognitive experience of emotion and to the hypothalamus where it acts to modulate the activity of the sympathetic and parasympathetic branches of the autonomic nervous system that are responsible for initiating visceral and skeletomuscular responses (Cannon, 1927; 1929; 1931; Bard, 1928). These visceral and skeletomuscular responses are what Cannon (1927; 1931) referred to as the flight and fight responses that protect an organism from danger and motivate it to search for a safe environment where food, water, shelter and a mate are available. Thus, the flight or fight responses and the highly charged emotional states instigated by the thalamus and hypothalamus were seen as being essential for survival.

Cannon (1928; 1929) and Bard (1928) based their views on studies demonstrating that emotional rage could still be elicited from cats after complete ablation of all brain matter anterior to the diencephalic region but leaving the thalamus intact (Cannon and Britton, 1925; Bard, 1928). However, when the lower posterior portions of the thalamus were removed in cats the emotionality as well as the accompanying muscular and visceral responses was attenuated (Cannon and Britton, 1925; Bard, 1928). Thus, the thalamus was viewed as the principal region of emotion because it was believed to act as a link between the cortical regions that provide the precise subjective and cognitive characteristics of an emotion and those brain regions that typically initiate the subsequent autonomic and behavioural changes associated with the emotion (Cannon, 1927; 1931; Bard, 1928).

It is important to point out that when discussing the role of the thalamus in emotion, Cannon (1931) was careful to make a clear distinction between the subjective emotional experience and the emotional behaviour (i.e. expression). Thus, according to Cannon (1931) the thalamus was not simply a seat of subjective emotionality, rather it was also an integrator of information and a discharger of emotional expression. In this sense the scale of the subjective emotional experience depends to a large degree on the integration processes taking place within the thalamus and on the precise areas of cortex that have been either recruited or inhibited (Cannon, 1931). According to this notion, spontaneous and inappropriate discharges of emotional behaviour are held in check by cortical inhibition of the thalamus and although some reactions can be attributed to thalamic output, the determining factors in whether or not a subjective experience of emotion occurs depends for the most part on intact thalamocortical connections and a properly functioning cortex

(Cannon, 1931). Based on his research, Cannon (1929;1931) went on to develop ideas on how the repeated transmission of nerve impulses through various thalamic pathways could lead to sensitisation and deep-seated changes in neural organization that facilitated learning, memory retrieval, emotional expression and even the reinstatement of emotional responses that were cortically suppressed (see Cannon, 1929). All that was required for emotional reinstatement (i.e. fear or rage expression) to occur was the presence of a sensitised set of responding neurons and salient cues and associations that could serve to trigger renewed responding (Cannon, 1929).

2.3: Emotion: From the Thalamus to the Hypothalamus and the Papez Circuit

As research into emotion continued experimental evidence obtained by Bard (1928), Ranson (1934), Hess (1936) and Bard and Rioch (1937) began to highlight the importance of the hypothalamus in mediating the expression of emotional responding. For example, Ranson (1934) discovered that electrically stimulating different regions of the hypothalamus in anesthetised animals caused significant alterations in heart rate, blood pressure and gastrointestinal responses that were similar in magnitude to responses produced during highly charged emotional states. In a similar vein, Hess (1936) consistently demonstrated that electrical stimulation of discrete nuclei in the hypothalamus of laboratory animals could elicit defensive or aggressive attack behaviours and flight reactions along with diffuse sympathetic activation typically observed during naturally occurring emotional reactions (also see; Hess, 1954). As a result, the hypothalamus began to displace the thalamus as the seat of emotions (see LeDoux, 1986 in LeDoux and Hirst, 1986; also see LeDoux, 1998).

From a historical perspective, the James-Lange (1890) and Cannon-Bard (1927; 1929; 1931; 1928) theories of emotion, coupled with Sherrington's (1906) work on modification of reflexes and Pavlov's (1927) research into classical conditioning were the first attempts to identify the neural mechanisms involved in emotional behaviour and how learned associations and representations of emotions are formed and represented in the central nervous system. This early research established the underpinnings for future research directed towards assessing the role of the central nervous system in emotion and emotionally related behaviours. More importantly, these early scientific efforts made it

possible to develop animal models of emotion and behaviour for a wide variety of emotions and behaviours found in humans, these include animal models of fear, anxiety, aggression, reward, and learning. This guided future researchers to manipulate various brain areas in several animal species (i.e. monkeys, cats, and rats) in order to explain how certain emotions are learned and expressed, and what specific brain areas are involved in mediating such emotions. This early work eventually set the stage for understanding how emotional responses may be learned and represented in the brain at the cellular and molecular levels.

In light of the research indicating that the hypothalamus was becoming an important area for the somatic and autonomic manifestations of emotion (Bard, 1928; Ranson, 1934; Hess, 1936; Bard and Rioch, 1937), James Papez (1937) attempted to delineate the precise neuroanatomical substrates and brain pathways involved in mediating emotional behaviour which centred on the hypothalamus. Papez (1937) identified the cerebral cortex and its substantial connections with several sub-cortical and limbic brain structures, including, the hippocampus, hypothalamus, mammillary bodies, fornix, anterior thalamic nuclei, and the cingulate gyrus and suggested that these areas may be the crucial anatomical substrates involved in mediating the emotional experience. Papez (1937) included these regions in his circuit of emotion because clinical observations of patients with damage to the cingulate cortex or hippocampus revealed that they were prone to display frequent episodes of intense emotional outbursts such as prolonged and uncontrollable laughter or crying (see LeDoux, 1998; LeDoux, 1986 in LeDoux and Hirst, 1986).

From an anatomical standpoint, this pathway, referred to as the "Papez Circuit", begins in the hippocampus and travels via the fornix to the hypothalamus and mammillary body. From the mammillary body, the pathway proceeds to the anterior thalamic nucleus, the cingulate gyrus, and innervates parts of the parahippocampal gyrus (i.e. the entorhinal cortex). From the parahippocampal gyrus the Papez Circuit then courses back to the hippocampus to complete the neural loop (Papez, 1937; Nolte, 1993).

Papez (1937) suggested that the hypothalamus receives both visceral and somatosensory information from a variety of peripheral sources and then via the mammillary bodies passes this information on to the cingulate gyrus. Thus, in this proposed model of emotion, the cingulate gyrus was considered to act as a sensory-emotional interface region that received

information from several cortical, subcortical and limbic structures (Papez, 1937). Since the cingulate gyrus was intimately connected to and interposed between several cortical and limbic structures, Papez (1937) believed that the cingulate acted as the primary cortical area involved in the reception of emotional information. It was hypothesized that the intimate connections in the Papez circuit made it possible for sensory processes occurring in the higher cortical regions to filter down and add emotional tone to an event or stimuli. Cortical projections to the hippocampus which in turn projected to the hypothalamus are thus able to influence or cause physiological and autonomic changes consistent with the behavioural expression of a particular emotion. Thus, Papez's (1937) model proposed that both the experience and expression of emotion was mediated by a highly integrated and intimately interconnected system that involves numerous limbic, subcortical and cortical brain areas each playing a specified role in processing information.

Papez (1937) envisioned that sensory input from the environment reaching the thalamus was relayed along separate pathways to three distinct brain areas. These areas include the hypothalamus, a region believed to be responsible for mediating feelings, the basal ganglia, believed to be responsible for initiating movement, and the cortex, believed to mediate thought processes and higher order functions (Papez, 1937). Papez (1937) focused most of his attention on the hypothalamic pathway responsible for mediating what he called "the stream of feeling". According to Papez (1937), sensory input entering the hypothalamus could be channelled in one of two directions. The first is towards the brainstem, spinal cord, and peripheral nervous system where the sensory stimuli could elicit autonomic and behavioural reactions without the recruitment or intervention of higher cortical regions like the sensory cortices which mediated the "stream of thought" (see more on Papez, 1937 in LeDoux and Hirst, 1986; LeDoux, 1998; Iversen, Kupfermann and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). The second is upward towards the cingulate cortex via its connections with the mammillary bodies and the anterior thalamic nucleus (Papez, 1937). In the cingulate cortex the sensory input combines and converges with information from other cortical areas responsible for processing auditory (e.g. temporal cortical area), visual (e.g. striate cortex), and somatosensory (i.e. parietal cortex) information, thus adding an emotional flavour to auditory, visual, and somatosensory impulses (Papez, 1937; also see LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998). This information is believed to

then be transferred to various cortical association areas, then on to the hippocampus, and finally terminates in the hypothalamus (Papez, 1937). The direction of the information flow, according to Papez (1937) allowed higher order cortical processes to influence and direct the hypothalamus to discharge the appropriate autonomic and behavioural responses typically associated with the specific emotion in question. This second sensory informational loop is what is commonly referred to as the "Papez Circuit".

Papez's (1937) ideas of the hypothalamus as a discharger of emotional information were undoubtedly influenced by Cannon's (1927; 1931) views on the thalamus acting as a discharger of emotion, however the bulk of Papez's ideas used to construct his theory of emotionality came from clinical observations of psychiatric patients and scant neuroanatomical information. Despite basing his views on limited anatomical observations and data it could be stated that Papez's (1937) model of emotion was nothing short of brilliant because it was not only parsimonious in the sense that it was able to maintain both the cortical and sub-cortical theories of emotion (i.e. attributing highly charged emotional expression to the hypothalamus and the subjective/cognitive experience of emotion to the neocortex), but it was also enduring and highly dynamic, in that it provided studies into emotionality with a neural circuit that could be scientifically evaluated and modified as new information became available (Papez, 1937; also see LeDoux, 1986 in LeDoux and Hirst, 1986; LeDoux, 1998). This to a large degree explains why Papez's (1937) model has stood the test of time and has been used so extensively to generate new models and theories about the neural circuitry and brain areas involved in mediating a variety of emotions such as aggression and fear.

2.4: From the Papez Circuit to the Limbic Theory of Emotion

As time went on, many scientific efforts designed to delineate the neuroanatomical pathways involved in emotion simply made additions or slight modifications to Papez's original circuit or were constructed out of his established theoretical framework. This evolutionary process ultimately led to the development of the limbic theory of emotion put forward by MacLean (1949; 1952). The limbic theory of emotion has dominated scientific thinking and inquiry for decades and to a large extent still remains one of the most influential theories about how the brain is involved in the formation of emotions. The

limbic system concept of emotion grew out of a context where science was employing and promoting an evolutionary explanation of the mind and behaviour (MacLean, 1949; 1952; 1958; 1970; Isaacson, 1982; LeDoux, 2000; LeDoux, 1998). As a result, the limbic theory of emotion was largely predicated on the views put forward by comparative neuroanatomists and physiologists who argued that the neocortex was a mammalian specialization and that cognitive processes such as thinking, reasoning, learning, memory, and problem solving must be mediated by the more evolutionarily advanced neocortex and not by older cortical, subcortical, or limbic-brain areas (MacLean, 1958; Isaacson, 1982; LeDoux, 1998; LeDoux, 2000). This notion is of course based on the premise that evolutionary based specialization and increased complexity is advantageous and helps the organism make adaptations that enable it to survive and flourish (for an alternative view on evolutionary theory and specialization see Gould and Norton, 1993; Gould, 2002). Thus, the paleocortical brain regions and related sub-cortical ganglia that together make up the limbic system were said to be responsible for initiating evolutionarily older aspects of behaviour and emotional reactions that are necessary for the survival of the organism and the preservation of the species (MacLean, 1949; 1952; 1958). As a result, cognition fell under the domain of the neocortex, whilst emotions and behaviours necessary for survival (i.e. fear, rage, escape and hunger) fell under the control of the evolutionarily older limbic system (MacLean, 1949; 1952; LeDoux, 2000).

It is quite clear that some of the ideas MacLean (1949; 1952) put forward concerning emotionality have been influenced by the work of both Cannon (1927) and Papez (1937). Thus, researchers working within this framework often concentrated their efforts on examining limbic brain regions thought to be involved in mediating such basic behavioural responses as feeding, fighting, fleeing, and sexual reproduction (i.e. reward) and the emotions associated with them (Hess, 1954; Kaada, Jansen and Andersen, 1953; MacLean, 1957; Miller, Bailey and Stevenson, 1950; Olds, 1954; 1955a; 1956b; 1956c; 1958; King, 1958a; 1958b). For example, the emotional circuit put forward by MacLean (1952) includes the septum, nucleus accumbens, amygdala, and hippocampus, as well as some neocortical areas such as the orbitofrontal cortex. MacLean (1952; 1958) referred to this circuit as the limbic system or visceral brain because these structures were involved in mediating autonomic functions and behavioural responses during emotionally arousing

situations that were in many instances designed for self-preservation. The hippocampus was especially important in MacLean's limbic model of emotion and MacLean (1949; 1952) saw the hippocampus as the site of visceral and sensory integration or what he referred to as "the seat of the emotional experience" (LeDoux, 1998).

MacLean (1949; 1952) based his views on the high degree of intimate connections that were observed between the hippocampus and the various cortical and sub-cortical structures, highlighting the fact that there is an overlapping of three main fibre systems entering the subiculum region of the hippocampus. In particular, MacLean (1949) attributed the longitudinal fibres linking the hippocampal formation along its entire rostral-caudal extent as possible regions that may be involved in the observed overlapping of various qualities (i.e. oral and sexual behaviour, auditory and visual hallucinations linked with fear and visceral fear and aggression) that contribute to an emotional experience. MacLean (1949) hypothesised that these anatomical characteristics make it possible for the hippocampus to act as an analyser that is capable of detecting, decoding, and picking out universal similarities from the particulars of a given experience and then relating them back symbolically to the experience of an emotion. MacLean (1949) went as far as suggesting that exaggerated, extreme, or dysfunctional forms of overlapping of qualities (i.e. oral and sexual behaviour, auditory and visual hallucinations linked with visceral fear and aggression) could explain why some psychotic patients display a bizarre mix of aggressive sadistic behaviour that is marked by sex, mutilation, disturbing hallucinations, paranoia and murder. Thus, MacLean (1949) attributed these extreme forms of emotionality to over-activity in the hippocampus and to a disconnection between the components of the visceral-brain (i.e. septum, nucleus accumbens, amygdala, hippocampus and orbitofrontal cortex) and the components of the word-brain located in the temporal, parietal and frontal cortices which make up Broca's and Wernicke's areas. MacLean (1949) based this view on the observation that patients with psychosomatic and psychotic mental illness generally have an inability to verbalize emotional feelings. As a result, rather than being directed to the intellectual word brain for further evaluation, the emotional feelings built up in the hippocampus find immediate expression through the limbic and autonomic centres responsible for discharging emotional behaviour (MacLean, 1949). Thus, MacLean (1949; 1952) felt that many behavioural manifestations and disorders of thought (i.e. oral fixations,

obsessive nail biting, hallucinations, paranoid ideations, hypersexuality, anxiety and self-mutilation) typically associated with psychiatric illness could be attributed to emotionally charged and adaptive behaviours produced in an overactive hippocampus and limbic system (i.e. visceral brain) that was for some reason disconnected from the inhibitory influence and executive control of the more highly developed neocortex (i.e. the word-brain) (LeDoux, 1998; 2000).

MacLean also based his theory on electrophysiological data making reference to the fact that electrical stimulation of the hippocampus produced after-discharge activity that was restricted to structures that made up the limbic system (MacLean, 1958). This evidence combined with the neuroanatomical results led MacLean (1949; 1952) to adopt the view that the role of the hippocampus was to sort out and correlate all internal and external sensory cues and then to pass this information on to the limbic system and other cortical structures in the brain. Thus, according to MacLean's (1958) limbic theory of emotion, the hippocampus was paramount, since it not only integrated internal and external information from the viscera and periphery but it was also involved in the recruitment of other limbic and cortical brain substrates involved in emotional expression. In essence, MacLean (1949; 1952; 1958) placed the hippocampus between the limbic structures involved in producing the raw emotional expression necessary for survival with those cortical brain areas that were capable of categorising, memorising and experiencing emotions subjectively. However, as time went on and as new research was introduced to the scientific community, the notion of the hippocampus being a key structure in the expression of emotion was to be seriously challenged (see LeDoux, 1998; 2000).

2.5: The Amygdala: "The Road Not Taken"

Historical Evidence Highlighting the Importance of the Amygdala in Fear and Emotion: "From the Hippocampus and Limbic Theory to the Amygdala and Emotional Circuits in the Brain"

The limbic theory of emotion based on the idea that the hippocampus was the seat of emotional experience began to wane as research demonstrated that damage to this structure caused severe deficits in cognitive functioning and long-term memory (Scoville and Milner, 1957). The problem facing the limbic theory was that hippocampal damage generally

produced profound deficits in declarative memory which relates to knowledge about people, places and things (Scoville and Milner, 1957; LeDoux, 2000). These functions were thought to fall under the domain of the more highly advanced neocortex and not under older and more primitive brain areas like the hippocampus. Although this was a significant blow to the limbic theory it certainly did not prove to be fatal as volumes of scientific journals and books were published extolling its virtues and its ability to explain almost every aspect of emotional behaviour (see LeDoux, 1998; LeDoux, 2000). Indeed, Gray's influential septo-hippocampal theory of emotion and anxiety grew out of the theoretical framework provided by the limbic theory and Gray's model does provide a detailed account of how some types of emotional behaviours are influenced by the septohippocampal neural system (for a review see Gray, 1982; Gray and McNaughton, 1983).

Another major problem that began to surface was that early neuroanatomical models of emotion that were based on the limbic theory often discounted the amygdala in playing a prominent role in the expression and experience of emotion (LeDoux, 1998; 2000). Often the amygdala was assigned a subordinate role when it came to explaining how emotional and behavioural processes associated with appetitive or aversive motivational stimuli were produced in the brain. Although, MacLean (1958) did acknowledge that the amygdala plays a role in mediating such self preservation behaviours as fear and aggression, he still maintained that the amygdala's functioning relied heavily on the functional integrity of the hippocampus. For example, MacLean (1949) envisioned the amygdala as a thalamus-like relay station that was largely responsible for processing olfactory stimuli and that an antagonistic relationship possibly existed between the amygdala and hippocampus given that the amygdala was observed to project almost exclusively to parasympathetic centres of the hypothalamus while the hippocampus targeted the sympathetic areas that were viewed to be involved in discharging the excitatory autonomic and behavioural responses associated with emotionality (MacLean, 1949). In this sense, the amygdala was assigned to an inhibitory role rather than to an excitatory role in emotional expression with the amygdala innervating the anterior hypothalamus and septal areas to control more placid viscerosomatic functions such as feeding, defecation and urination (MacLean, 1949). Thus the amygdala's role in mediating such highly charged emotional states as fear and anxiety were not fully recognized and the amygdala was relegated to a subordinate role as far as

emotion and emotional expression was concerned. This view prevailed for some time despite the fact that some earlier research had already demonstrated that damage to the temporal lobe region that included the amygdala could produce profound deficits in the emotional expression of fear (Brown and Schäffer, 1888 in LeDoux, 1998; 2000; Klüver and Bucy, 1939).

In fact, the earliest scientific evidence implicating the amygdala in emotion and fear expression was in experimental work conducted by Brown and Schäffer (1888). These researchers found that temporal lobe lesions carried out on wild monkeys, transformed the normally fearful and cautious primates into tame and indifferent animals that seemed to show no visible signs of fear. A few decades later, Klüver and Bucy (1939) discovered that bilateral ablations of the anterior temporal lobe caused several behavioural changes and deficits in monkeys. These deficits included uncharacteristic tameness, increased sexual activity, visual agnosia (psychic blindness), lack of fearfulness of objects that normally caused fearful reactions, and hypo-emotionality. This pattern of significant behavioural change observed in monkeys following temporal lobe ablations became known as the Klüver-Bucy syndrome. Similar Klüver-Bucy like behaviours were also produced in human clinical populations following amygdaloid and temporal lobe removal as a treatment for intractable psychotic behaviour, hyper-emotionality, and epilepsy, however it must be noted such symptomatology was only observed when the amygdala and surrounding cortical tissue was removed (Obratdor, 1947; Green, Duisberg, and McGrath, 1951; Scoville, Dunsmore, Liberson, Henry and Pepe, 1953;Williams, 1953; Sawa, Ueki, Arita, and Harada, 1954; Pool,1954; Terzian and Ore, 1955). Furthermore, it is important to highlight the fact that some emotional and psychotic abnormalities remained after surgical removal of the amygdala (Freeman and Williams, 1952; Scoville et al., 1953; Sawa, et al., 1954; Scoville and Milner, 1957) but this may be more a function of the surgical technique used and the actual degree of amygdaloid damage produced.

Around the same time that the limbic theory of emotion was being developed and placed under scientific scrutiny, Weiskrantz (1956) and Downer (1961) conducted experiments on monkeys to determine the specific brain structure in the anterior temporal pole that caused the Klüver-Bucy syndrome. These researchers found that lesions confined to the medial temporal lobe or amygdala caused the behavioural deficits associated with Klüver-Bucy

syndrome (Weiskrantz, 1956; Downer, 1961). Perhaps the most dramatic change in monkeys after amygdaloid removal was a profound loss of emotional responsiveness combined with a general willingness to approach and examine previously frightening stimuli (i.e. snake) without any observable fear reaction or cautious behaviour (Walker, Thompson, and McQueen, 1953; Weiskratz, 1956; Downer, 1961). This scientific evidence demonstrated that the amygdala might play an even more prominent role in emotionally motivated behaviours than the hippocampus. This notion was further supported by clinical assessments made of aggressive and hyperactive patients that received stereotaxically induced amygdalotomies to treat their behavioural and emotional outbursts (Narabayashi, Nagao, Saito, Yoshida and Naghata, 1963). These assessments found that bilateral or unilateral removal of only one-third of the amygdaloid complex was sufficient to cause eighty-five percent of the subjects to display marked reductions in emotional excitability (Narabayashi, et al., 1963). Many of the patients were reported to have more normalized social behaviour and only became fearful, aggressive, and excitable when appropriate or when repeatedly provoked (Narabayashi, et al., 1963).

Further evidence for a more prominent role of the amygdala in fear and emotionality comes from clinical research on humans suffering from intractable temporal lobe epilepsy. Most epileptic seizure activity begins in the amygdala and usually spreads into adjacent temporal lobe areas (Gloor, 1992). Past research along with more current research has consistently found that ictal fear is the most common emotion associated with temporal lobe epileptic discharge (Macrae, 1954; Penfield and Jasper, 1954; Williams, 1956; Daly, 1958; Gibbs, 1956; Mullan and Penfield, 1959; Weil, 1959; Gloor, 1972; 1990; 1991; Strauss, Risser, and Jones, 1982; Talyor and Lochery, 1987; Wieser, 1983). This ictal fear state can be characterized by fearful hallucinations, frightening memory flashbacks, and feelings of intense terror that are at times associated with heart palpitations, mydriasis (i.e. pupil dilation), and epigastric upset. Furthermore, electrical stimulation of the amygdala or temporal lobe region in people with epilepsy has been shown to reliably reproduce many of the physiological and fear-like emotional states that occur during full epileptic seizures (Penfield and Jasper, 1954; Jasper and Rasmussen, 1958; Mullan and Penfield, 1959; Gloor, 1972; Halgren, Walter, Cherlow, and Crandall, 1978; Gloor, Oliver, and Quesney, 1981). It is noteworthy that many of these fear-like states produced by amygdaloid

stimulation seemed to tap into or somehow reactivate real fear memories that may have been caused by the patient who actually experienced a traumatic event in the past or simply read about one in a novel or watched too many horror movies. Thus, whether or not the fear memory is based on a real experience or produced through imagination or some other source, amygdaloid stimulation seems to revive them.

It is important to note that electrical stimulation of either the hippocampal formation or cortical regions distal to the amygdala generally does not reproduce these profound fear-like states in epileptic patients (Gloor, 1972; 1992). Thus, ictal fear seems to occur only when the after discharge activity produced in other stimulated regions reaches the amygdala and induces amygdaloid neuronal excitation (Gloor, 1972; 1992). This indicates that the amygdaloid neural activity plays a prominent role in mediating both the physiological and subjective emotional states associated with fear. For example, clinical patients that have received bilateral amygdalectomies to help alleviate the behaviours associated with temporal lobe epilepsy show marked deficits in recognizing facial expression of fear (Adolphs, Tranel, Damasio and Damasio, 1994; 1995). These subjects also have difficulty recognizing the emotional tone of sentences when they are read in a fearful voice, however, declarative memory, verbal skills and recognition of familiar faces is left intact (Scott, Young, Calder, Hellawell, Aggleton and Johnson, 1997; Adolphs, et al., 1995). Similarly, subjects with unilateral temporal lobectomies that produced damage to the amygdala and parts of the hippocampus were reported to display significant impairments on both simple and conditioned discrimination tasks (LaBar, LeDoux, Spencer and Phelps, 1995). In general, these subjects demonstrated an inability to acquire conditioned fear responding, however their verbal abilities and declarative knowledge was intact (LaBar, et al., 1995). These results indicate that the amygdala adds an emotional flavour to memories and events that normally fall under the domain of supposedly more highly advanced brain regions such as the hippocampus and neocortex. It is therefore likely that the amygdala plays a key role in the acquisition, storage, and retrieval of fear evoking emotional memories and possibly adds emotional content to memories stored elsewhere.

The discovery that humans show deficits in fear expression following the surgical removal of the amygdala closely paralleled animal research which also demonstrated that

amygdaloid lesions produce marked impairments in fear-motivated behaviours and avoidance responding. For example, in terms of conditioned fear, several studies that measure active avoidance responding as a behavioural correlate of fear consistently showed that animals with amygdala lesions exhibit marked impairments in this task (Brady, Schreiner, Geller and Kling, 1954; Horvath, 1963; Ursin, 1965; Thatcher and Kimble, 1966; Coover, Ursin and Levine, 1973; Goldstein, 1974). Additionally, complete destruction of the amygdala or lesions confined to discrete amygdaloidal nuclear regions caused impairments in passive avoidance behaviour (Ursin, 1965; Pellegrino, 1968; Slotnick, 1973; Grossman, Grossman and Walsh, 1975; Russo, Kapp, Holmquist and Musty, 1976) and defensive freezing (Blanchard and Blanchard, 1972). As more and more research implicating the amygdala's role in mediating both conditioned and unconditioned fear emerged (Gloor, 1960; Goddard, 1964; Blanchard and Blanchard, 1972; Cohen, 1975; Kapp, Frysinger, Gallagher and Haselton, 1979; Cohen, 1974) the limbic theory was forced to reconsider its position regarding the role of the hippocampus in emotionality and to change with the times.

The limbic theory of emotion has evolved considerably during the five decades since its inception and has finally given the amygdala a much greater status as far as the study of emotion is concerned, however this has taken a long time and has been a difficult journey. A great deal of this difficulty arose as new brain areas were simply added to the limbic system without having a clearly defined criteria established (LeDoux, 2000). Even today there is still no agreed upon criteria amongst neuroscientists for including a specific brain area into the limbic system's emotional hall of fame. In some instances, all that is required for inclusion into the limbic system is that a brain area either enhances or inhibits some small aspect of emotional behaviour or shares connections with brain nuclei that already belong to the limbic system (LeDoux, 2000). This made it difficult for the amygdala to emerge out of the shadows and gain recognition as a key player in the emotion game. As such, the amygdala was often lost in the shuffle of an ever expanding limbic system. As LeDoux, (2000) points out, this problem was further compounded by apathetic researchers who were on an insistent quest to find a brain area that corresponded to every minute detail of a particular emotion being studied.

Another difficulty facing the limbic system theory of emotion is that the evolutionarily-based lines of distinction between functions that fall under the domain of the old brain (limbic/visceral brain) versus those that fall under the new brain (neocortex/word brain) have either broken down very early on when the theory was introduced or have become very blurred as new research has come to light (LeDoux, 2000; LeDoux, 1998). Nowhere was this difficulty for the limbic theory made more apparent than in the discovery that bilateral hippocampus lesions produced a severe impairment in a cognitively based long-term memory task that was for the most part emotionally neutral (Scoville and Milner, 1957; LeDoux, 2000; 1998). The shortcomings of the limbic theory became even more pronounced as research evidence implicating the amygdala's role in mediating fear-motivated behaviours and conditioned fear came to light (Brady, Schreiner, Geller and Kling, 1954; King, 1958a; 1958b; Horvath, 1963; Ursin, 1965; Thatcher and Kimble, 1966; Weiskrantz, 1956; Downer, 1961; Gloor, 1960; Goddard, 1964; Blanchard and Blanchard, 1972; Cohen, 1974).

Still more difficulties arose as the limbic system continued to expand from its original form of a paleocortex that contained just a few subcortical structures, to a larger and more advanced hybrid version that added several midbrain and neocortical areas to the original mix (Nauta, 1979; Kaada, 1960; LeDoux, 2000; LeDoux, 1998). The poorly operationally defined criteria for inclusion of a brain area into the limbic system frame work, combined with an ever expanding limbic system, made the study of emotions very difficult, because any new brain area that contributed to any facet of an emotion either simply validated the entire limbic system concept or provided a reason to add another brain area to the limbic circuit (LeDoux, 2000; LeDoux, 1998). As a result, more research energy was put into pursuing experiments that validated the limbic system, and as a consequence, less focus was placed on how a specific emotion such as fear might be the end product of a specific neural circuit operating within the limbic system (LeDoux, 2000). In essence, the limbic theory may have made the unfortunate error of applying a Gestalt approach to the study of emotion and fear (i.e. focusing on the whole limbic brain and emotionality rather than the sum of the parts that contribute to a particular emotion). As mentioned above, this made it difficult for the amygdala to gain any recognition as a brain area that might be responsible for mediating such emotional responses as fear and aggression. These problems have led

some researchers to suggest that the limbic theory of emotion is somewhat antiquated and that the concept of a limbic theory should be revamped or simply abandoned (LeDoux, 1991; Kotter and Meyer, 1992; LeDoux, 2000; LeDoux, 1998).

Despite some of the difficulties with the limbic theory, it is generally accepted that this theory did in the end leave the research community with at least two important legacies that can be employed to unlock the mysteries surrounding the brain areas that are involved in mediating emotional behaviour and fear learning (see LeDoux, 2000; LeDoux, 1998). The first is the notion that cognition, which involves declarative memory and higher order sensory information processing, might recruit or employ different neural circuitry than emotional circuits, and these may for the most part function relatively independent of each other and have limited overlap (LeDoux, 2000; LeDoux, 1998). The second, and perhaps more important legacy, is the view that emotions such as fear and aggression which are necessary for self-preservation involve relatively primitive neural substrates and circuits that have been maintained throughout evolution (LeDoux, 2000; LeDoux, 1998).

In general these two legacies incorporate MacLean's (1949; 1952) idea that there is usually some degree of separation between the limbic-brain which initiates highly charged emotional responses in an automatic and unconscious fashion and the word-brain which is involved in cognitive and conscious processing. If and when overlap between the two brain systems does occur, two important things may happen. First, emotional tone or flavour is added to cognitively processed events and memories which enhance the subjective experience of emotions such as fear. Second, emotionally charged fear memories may also be stored and consolidated in certain regions of the neocortex which possibly adds a complex cognitively based structure to the fear memories stored in limbic areas like the amygdala. This seemingly redundant process may enhance the response capacity of the emotional systems and provide an organism with the necessary resources to learn, avoid danger, and survive. Taken together, these ideas led to the development of theoretical models that have embraced the notion that fear learning and expression involves distinct neural substrates and emotional circuits in the brain (LeDoux, 2000; LeDoux, 1998) and many of these circuits have the amygdala located at their core (for reviews see LeDoux, 1993; 1998; Davis, 1992a,b,c; Davis, 2000; Maren, 1996; 1999; Fendt and Fanselow, 1999).

Today most scientific and biopsychological theories of emotion implicate the amygdala as a key limbic brain area in mediating both conditioned and unconditioned fear behaviours and as a possible site for the learning, storage, consolidation, and retrieval of fear memories (for reviews see, Blanchard and Blanchard, 1972; Cohen, 1975; Kapp, Frysinger, Gallagher and Haselton, 1979; Aggelton and Miskin, 1986; LeDoux, 1987; LeDoux, 1992; 2000; 2002; Davis, 1992; 1997; 2000; Fanselow and LeDoux, 1999; Maren, 1999; Davis 2000, LeDoux, 2000; Fendt and Fanselow, 1999; Schafe, Nader, Blair, and LeDoux, 2001; Stork and Pape, 2002; Schafe and LeDoux, 2000). An alternative view found in the research literature suggests that the amygdala only plays a temporary role in the consolidation of fear-memories and that these memories are not encoded within the amygdala's neurocircuitry (see Cahill and McGaugh, 1999; Cahill, Weinberger, Roozendaal, and McGaugh, 1999; Weinberger, 1998). Some researchers even question if the amygdala is a locus of conditioned fear and dispute its involvement in the rapid acquisition of classically conditioned fear responses (Cahill, Vazdarjanova, and Setlow, 2000; Cahill, et al., 1999). Despite this difference of scientific opinion, it is possible that the amygdala initiates neurobiological actions that facilitate both the local storage and consolidation and the remote storage and consolidation of fear-memories that occur during aversive classical conditioning, thus ensuring that redundancy is built into the amygdala-based fear neurocircuitry after conditioning takes place (Maren, 2001).

The amygdaloid complex is a unique brain area consisting of several intimately interconnected and multilayered cell-rich nuclei that together receive a wealth of afferent information from virtually every cortical and subcortical structure in the brain. The amygdala also sends efferent projections back to many of these cortical and subcortical structures, thereby placing it in a strategic position that would allow it to influence the acquisition and expression of emotional behaviours and to integrate, process, store, and retrieve information that concerns appetitive or aversive motivational stimuli. In a general sense, the amygdala and its intimate connectivity with other brain areas can be viewed as an important emotional neural circuit or substrate in the brain that not only acquires and expresses fear behaviours but also involves itself with memory storage and retrieval processes that add emotional valence to sensory specific cues or environmental stimuli. In order to get a greater appreciation of the amygdala's role in mediating fear behaviours it is

necessary to consider the anatomy, connectivity, neurochemistry and cellular morphology of the amygdala in more detail.

Chapter 3

Looking Inside the Black Box: The Anatomy, Connectivity, and Cellular Morphology of the Amygdala

The amygdala is composed of approximately twelve distinct regions or nuclei, each of which can be further divided into several subregions or subnuclei often referred to as subdivisions (McDonald, 1992; 1998; Cassell, Freedman and Shi, 1999; Pitkänen, 2000; LeDoux, 2000). The boundaries between these amygdaloid nuclei are largely based on the cytoarchitectonic and histochemical staining properties of the neurons occupying a given region and on the connectivity of these neurons with other brain regions (Amaral, Price, Pitkänen, and Carmichael, 1992; McDonald, 1992; 1998; Alheid, de Olmos, and Beltramino, 1995; Pitkänen, 2000). Given the amygdala's complex cellular organization and its vast connectivity, it is helpful to provide an anatomical description of each individual nucleus and their position in relation to each other, along with a brief but detailed account of each nucleus's cellular composition, organization, immunohistochemical properties and connectivity with other brain regions. For the most part these discussions will rely more heavily on scientific investigations that have been carried out on the rat brain as they are the species most often used in experimentation, however some references will incorporate anatomical research conducted on mammals that rank higher on the phylogenic scale such as the cat and monkey.

3.1: Three General Groups of the Amygdala

Based on findings obtained from cytoarchitecture, histochemical, Golgi and Nissl staining, anterograde and retrograde tracing studies and electromicroscopic analysis, the amygdaloid nuclei can be divided into at least three general groups (McDonald, 1998; Cassell, et al., 1999; Savander, Miettinen, LeDoux, and Pitkänen, 1997; Pitkänen, 2000). First there are the deep amygdaloid nuclei which include the lateral, basal, and accessory basal nuclear groups which collectively make up what is referred to as the basolateral nuclear group (Amaral, et al., 1992; McDonald, 1998; Pitkänen, 2000). Second there are the superficial nuclei and areas which include the anterior cortical nucleus, the posterior cortical nucleus, the medial nucleus, the nucleus of the lateral olfactory tract and the

periamygdaloid cortex. These amygdaloid nuclei are also referred to as "superficial cortex-like nuclei" because their neuronal architecture is similar to that of the laterally adjacent olfactory cortex and due to their close proximity to the surface of the brain (McDonald, 1998; Pitkänen, 2000). Finally, there are the central nucleus and the remaining amygdaloid nuclei which include the anterior amygdaloid area, the amygdalohippocampal area (AHA) and the intercalated nuclei (Amaral, et al., 1992; McDonald, 1998; Pitkänen, 2000). This group is referred to as the centromedial nuclear group and is located in the dorsomedial aspects of the amygdala in all mammalian species including the rat (McDonald, 1998; Cassell, et al., 1999; Pitkänen, 2000). According to McDonald (1998), this group also includes the medial amygdaloid nucleus along with the bed nucleus of the stria terminalis (BNST) which is considered by some to be a rostral extension of the centromedial nuclear group (Johnston, 1923; Alheid, De Olmos, and Beltramino, 1995; McDonald, 1998).

It is important to note, that McDonald (1998) includes the medial amygdaloid nucleus in the centromedial nuclear group, whereas Amaral, et al., (1992) placed the medial nucleus into the superficial amygdaloid nuclear group. To avoid ambiguity, this thesis will follow the convention put forward by McDonald (1998) and place the medial nucleus and the BNST into the centromedial nuclear group. It is helpful however, to be aware of discrepancies in amygdaloid terminology, nomenclature, and categorization, as they typically arise as researchers study different mammalian species (i.e. cat, rat and monkey) in their quest for greater understanding of amygdaloid function and connectivity. Moreover, it is important to become acquainted with the cell types and the neurotransmitter and neuropeptide receptors sites found in various amygdaloid regions as this information will be valuable to the reader when later chapters review experiments that pharmacologically manipulate the amygdala and successfully block conditioned fear.

3.11: The Deep Amygdaloid Nuclei

The deep nuclei of the amygdala include the lateral nucleus, the basal nucleus and the accessory basal nucleus. In the rat, the lateral amygdaloid nucleus is bordered laterally by the external capsule and ventromedially by the basal nucleus (McDonald, 1998; Savander, et al., 1997; Pitkänen, et al., 1995; Pitkänen, 2000). The lateral nucleus is further subdivided into three distinct subnuclei, these include, the dorsolateral, ventrolateral, and

ventromedial divisions (McDonald 1998; Amaral, et al., 1992; Savander, et al., 1997; Pitkänen, 2000). The basal nucleus is located just ventromedial to the lateral nucleus and has two major subdivisions which are the magnocellular basal nucleus and the parvicellular basal nucleus (Krettek and Price, 1978a,b; McDonald 1998; Savander, et al., 1997; Pitkänen, 2000). Ventromedial to the basal nucleus is the accessory basal nucleus which is bordered by the more ventral and laterally positioned paralaminar amygdaloid nucleus. This thin zone of neurons in the paralaminar nucleus encapsulates the entire basolateral nuclear group along its ventral and lateral aspects (McDonald 1998).

Cell Type and Structure

Nissl and Golgi staining techniques in the rat and cat have demonstrated that the cells of the basolateral amygdaloid nuclei are similar in structure and composition to cells found in the piriform cortex, hippocampal formation, claustrum and cerebral cortex (McDonald 1992; Gurdjian, 1928; Hall 1972). Extensive staining studies (Gurdjian, 1928; Hall 1972; Alheid, et al., 1995) in combination with more recent experiments incorporating neurotransmitter immunohistochemical analysis and electron microscope techniques (McDonald 1992; Roberts, 1992; Carlsen and Heimer, 1988; Savander, et al., 1997) indicate that the neurons of the basolateral complex fall into two major cellular classes, these are the spiny pyramidal-like neurons (type P cells) and the spine-sparse stellate neurons (type S cells). Hall (1972) and various other researchers (McDonald, 1992; 1998; Roberts, 1992; Carlsen and Heimer, 1988; Alheid, et al., 1995; Savander, et al., 1997; Pitkänen, et al., 1995; Pitkänen, 2000) have noted that the pyramidal and nonpyramidal neurons of the basolateral complex closely resemble their counterparts in the cerebral cortex, with the only observable difference being that the main apical dendrites of amygdaloid pyramidal neurons are not arranged in a parallel fashion to each other (McDonald, 1998; Pitkänen, 2000).

Pyramidal Neurons

McDonald (1992) notes, that the pyramidal neurons of the basolateral nuclear group are characterized structurally by a dense covering of dendritic spines located on secondary dendrites and more distal dendritic branches that are situated further away from the cell

body. Typically, each of these dendritic spines has a prominent distension that is located on the terminal region of the spine (McDonald, 1992). This terminal swelling on the spine begins to narrow into a thin stalk as it comes into contact with the dendritic branch. Hence, each dendritic spine has the general shape of a light bulb, with a thin stalk (1.0- 1.5 μm long) contacting a dendritic branch and a swollen terminal region that contains numerous receptors that are involved in neurochemical processes that enhance synaptic transmission.

Like their cortical counterparts, pyramidal amygdaloid neurons are not all the same, and do indeed show a great deal of variability in terms of size and structural characteristics. For example, at one end of the cellular spectrum the amygdaloid pyramidal neurons are almost identical to cortical pyramidal neurons, while at the other end of the spectrum they closely resemble cortical spiny stellate cells (McDonald, 1992). Moreover, ultrastructural, synaptological (Lund, 1984; Saint Marie and Peters, 1985) and electrophysiological studies (Conners and Gutnick, 1990) of cortical pyramidal and stellate cells along with immunohistochemical experiments carried out on amygdaloid neurons (McDonald, Beitz, Larson, Kuriyama, Sellitto and Madl, 1989; McDonald, 1989) indicate that the pyramidal amygdaloid neurons may form part of an array of spiny cortical-like neurons. Interestingly, these amygdalar neurons, like their counterparts in the cerebral cortex show intense immunoreactivity for glutamate and aspartate (McDonald et al., 1989; McDonald, 1989; LeDoux and Farb, 1991), both of which are necessary prerequisites for the induction of LTP processes required for learning and memory storage. Indeed the high levels of binding demonstrated with ligands for N-methyl-D-Aspartate (NMDA), kainate, and quisqualate (Monaghan and Cotman, 1985; Miller, Johnson, Gelhard and Insel, 1990; Insel, Miller, and Gelhard, 1990; McDonald, 1994; Farb, Aoki and LeDoux, 1995) indicate that the amygdala has a large population of NMDA and AMPA kainate receptors that may be involved in LTP and synaptic transmission processes deemed necessary for fear acquisition and expression (see Davis, Rainnie and Cassell, 1994; Farb, et al., 1995; Rainnine, Asprodini, and Shinnick-Gallagher, 1991a; Maren, 1996; Wang, Wilson, and Moore, 2001; McKernan and Shinnick-Gallagher, 1997; Mahanty and Sah, 1998; Fendt, 2001).

Non-Pyramidal Neurons

The nonpyramidal neurons of the basolateral complex typically contain spine-sparse dendrites and have been observed to be dispersed among the pyramidal neurons in this region (McDonald, 1992; Pitkänen, 2000). The axons of nonpyramidal neurons emit several beaded collaterals and these form fairly substantial axonal arborization in the vicinity of the parent cell and also frequently form delicate synaptic contacts with the dendrites of pyramidal neurons (McDonald, 1982a; McDonald, 1982b; Millhouse and De Olmos, 1983). The nonpyramidal cells are a heterogeneous population consisting of multipolar, bitufted, and bipolar cell types with several specific subpopulations based on distinct axonal or dendritic features (McDonald 1982b; McDonald, 1992).

The most common nonpyramidal neurons with distinctive dendritic features are the amygdaloid chandelier cells, neurogliaform cells, and cone cells. Chandelier cells form intimate connections with axonic spines of several amygdaloid pyramidal neurons (McDonald and Culberson, 1981; McDonald, 1982b) and since experimental evidence suggests that similar cells in the cerebral cortex are inhibitory (Peters, 1984), it is possible that amygdaloid chandelier cells could exert a potent inhibitory influence on amygdaloid pyramidal cells. Since the initiation of action potentials in pyramidal neurons depends to a large degree on the spatial and temporal summation of excitatory post synaptic potentials arriving via the axonic and dendritic spine route, the inhibitory (i.e. hyperpolarizing) effects of just one chandelier cell could significantly reduce the firing rates of numerous pyramidal cells. This process is often referred to as "the sculpturing role of inhibition" (Kandel and Siegalbaum, 2000 in Kandel, Schwartz and Jessell, 2000 pg 209).

It is important to note that many of the nonpyramidal neurons in the basolateral complex contain high cellular and terminal levels of GABA, choline acetyltransferase, calcium binding proteins, and a vast array of peptides, including somatostatin, cholecytokinin (CCK) and neuropeptide Y (McDonald, 1984; McDonald, 1985a; McDonald, 1985b; McDonald and Baimbridge, 1990; Carlsen and Heimer, 1986; Gustafson, Card, and Moore 1986; McDonald and Pearson, 1989). Taking these facts into account, it is highly probable that these nonpyramidal neurons may be able to influence the activity of pyramidal cells in the region, inhibit synaptic transmission, disrupt LTP, and thus alter amygdaloid output (Rainnie, Asprodini and Shinnick-Gallagher, 1991b). Glutamic acid decarboxylase (GAD)

and/or GABA-immunoreactive cell bodies are dispersed widely throughout all amygdaloid nuclei, with higher concentrations located in the lateral, basal, and cortical nuclei (McDonald, 1985b; Ottersen, Fischer, Rinvik and Storm-Mathisen, 1986; Nitecka and Ben-Ari, 1987; McDonald and Pearson, 1989 Rainnie, et al., 1991b). This is particularly important for understanding some of the neurobiological mechanisms involved in fear and anxiety, since the anxiolytic action of benzodiazepines are mediated by their selective binding with benzodiazepine receptor sites that interact allosterically with the $GABA_A$ receptor complex (Costa, Guidotti, Mao, and Suria, 1975; Richards and Möhler, 1984). This may explain why both benzodiazepines and $GABA_A$ agonists are so effective at inhibiting fear and anxiety in both humans and animals (for a review see Blanchard, Griebel, Rodgers, and Blanchard, 1998; Clement and Chpouthier, 1998; Drugan and Holmes, 1991; Lader, 1994; File, 1987; Menard and Triet, 1999; Davis, 1979; Fanselow and Helmstetter, 1988).

Amygdaloid neurogliaform neurons also send abundant axonal collaterals as well as distal dendritic branches to form numerous contacts with dendrites of neighbouring amygdaloid pyramidal cells (McDonald, 1992) however this dense tangle of collaterals only extends slightly beyond the confines of the neurogliaform's own dendritic field. As such, these small spherical-shaped cells appear to be more typical of local circuit neurons that have a very confined sphere of influence (McDonald, 1992). In contrast, cone cells located in the lateral nucleus (Millhouse and De Olmos, 1983) typically have large cell bodies and spine-sparse dendrites that generate abundant and irregular varicosities. These cells have appropriately been classified by McDonald (1992) as large nonpyramidal cells.

Finally, it is important to point out that an abundant population of GABAergic interneurons reside in the basolateral complex and many of these receive excitatory input mediated by the unedited subunit of calcium-permeable AMPA receptors known as GluR2 (McDonald, 1985b; McDonald and Pearson, 1989; Mahanty and Sah, 1998). Research has shown that tentanic stimulation of synapses that impinge on these interneurons leads to AMPA receptor-mediated LTP that is triggered by the increase of postsynaptic calcium entering through the ion channels of the AMPA receptors (Mahanty and Sah, 1998). The repeated and increased excitation of the GABAergic interneurons in the basolateral complex causes numerous inhibitory potentials to occur in many of the pyramidal cells of

this region, and it has been suggested that this increase in inhibitory synaptic transmission may be responsible for the increased synchronization and desynchronization of firing activity that typically occurs between neurons in the basolateral amygdala (BLA) after Pavlovian fear conditioning (see Quirk, Repa, and LeDoux, 1995; Mahanty and Sah, 1998).

3.12: The Superficial Cortex-Like Amygdaloid Nuclear Group

To briefly recap, the superficial cortex-like amygdaloid nuclear group contains the anterior cortical nucleus, the posterior cortical nucleus, the nucleus of the lateral olfactory tract and the periamygdaloid cortex. As stated earlier, some researchers choose to include the medial nucleus of the amygdala into the superficial cortex-like category (Amaral, et al., 1992) however, since this thesis is following the categorization scheme of McDonald (1992-1998) most discussions pertaining to the medial amygdaloid nucleus will be found in next section which deals with the centromedial nuclear group. Nevertheless, some references to the medial amygdaloid nucleus will be made here, but the discussions will only use the medial nucleus as an anatomical reference point to help localize the nuclei of superficial cortex-like group and demarcate nuclear boundaries.

The very anterior subdivision of the cortical nucleus and the nucleus of the lateral olfactory tract form the anterior border of the superficial cortex-like amygdaloid nuclear group (McDonald, 1992; 1998; Pitkänen, 2000). In the rat brain, at the most anterior level the anterior cortical nucleus is located lateral to the nucleus of the lateral olfactory tract and is bordered on its lateral edge by the cortical amygdaloid transition zone and the piriform cortex. More caudally, the anterior cortical nucleus is bordered dorsally by the anterior portion of the basomedial nucleus and mediolaterally by the anterior portions of the medial amygdaloid nucleus. At its lateral-most edge the anterior cortical nucleus is bordered by the piriform cortex (McDonald, 1998; Alheid, De Olmos and Beltramino, 1995; Paxinos and Watson, 1986).

The posterior subdivision of the cortical nucleus forms the most caudal component of the superficial cortex-like nuclear group (McDonald, 1998) and is bordered dorsolaterally by the piriform cortex and intracalated amygdaloid nuclei (Alheid, et al., 1995; Pitkänen, 2000; Paxinos and Watson, 1986). Medially, the posterior cortical nucleus is bordered by the posteroventral portion of the medial amygdala. Dorsally, the posterior cortical nucleus

eventually forms the ventral borders of the basolateral, basomedial, and posterior basolateral amygdaloid nuclei when moving in a rostrocaudal direction (Alheid, et al., 1995). Finally, the periamygdaloid cortex (PAC) is located primarily at intermediate levels of this group and consists of various subdivisions in the rat and monkey (McDonald, 1998; Amaral, et al., 1992 in the Amygdala). Price, Russchen, and Amaral (1987) subdivided the monkey periamygdaloid cortex into four subdivisions called PAC1, PAC2, PAC3, and PACs, whilst Paxinos and Watson (1986) refer to this area in the rat as the posterolateral subdivision of the cortical nucleus (McDonald, 1998; Alheid, et al., 1995). In the rat, the periamygdaloid cortex is located ventral to the accessory basal amygdaloid nucleus (McDonald, 1998).

Cytoarchitecture and Cell Type

Each of the amygdaloid nuclei in the superficial cortex-like nuclear group contains at least three and in some cases, depending on the species studied, even four distinct cellular layers (McDonald, 1998; McDonald, 1992; Hall, 1972; Alheid, et al., 1995). Layer I usually consists of a cell sparse molecular layer with fibres from olfactory bulbs passing through the area. Layers II , III or in some cases IV, are the cell dense layers with a clear laminar configuration and distinct boundaries, however the extent to which different laminae are recognizable varies to a large degree on the amygdaloid nuclei being studied and the level at which brain slices were taken (McDonald, 1992;1998; Alheid, et al., 1995). This identification process is made even more complicated if slices are taken in transition areas between two distinct nuclei, thus making it difficult to identify both laminae and nuclear boundaries (Alheid, et al., 1995).

Pyramidal Neurons of the Amygdaloid Cortical Nucleus

Most of the neurons in the cortical nucleus (which also includes the periamygdaloid cortex), (for a review see McDonald, 1998) are of a spiny pyramidal and semipyramidal variety, similar to the spiny pyramidal neurons (type P cells) found in the basolateral amygdaloid complex (McDonald, 1992; 1998; Pitkänen, 2000). These neurons and especially those located in the more superficial nuclei have clear and well defined apical dendrites that are oriented perpendicular to the brain surface (McDonald, 1998). The spiny

pyramidal neurons in the cortical nucleus are found primarily in the cell dense layers II to IV and they send their apical dendrites into the superficial plexiform layer, however some do send basalar dendrites into the cell sparse Layer I (McDonald, 1992). Additional subgroups of pyramidal neurons that have prolific apical and basal dendrites are also present in the cortical nucleus and thus may act as projection neurons. These are referred to as the modified pyramidal cells, and in some instances these neurons exhibit polygonal or fusiform configurations (McDonald, 1992). The axons of these spiny neurons innervate the stria terminalis and give off a series of collaterals that breeches the deeper portions of the targeted area (McDonald, 1992), raising the possibility that the pyramidal neurons of the amygdaloid cortical nucleus likely transmit sensory information to the septum and hypothalamus via the rostral amygdalofugal pathway that runs through the stria terminalis (Krettek and Price, 1978a).

Non-Pyramidal Neurons of the Amygdaloid Cortical Nucleus

Another major cell type located in the rat cortical nucleus is the spine-sparse nonpyramidal cells with their characteristic beaded dendrites (McDonald, 1978; McDonald, 1992). These neurons are also very similar to the spine-sparse cells found in the BLA, and their axonal arborization is found near the cell of origin which indicates these neurons are most likely local circuit neurons (Szafranska-Kosmal and Tömböl, 1972; Kamal and Tömböl, 1975; McDonald, 1992; Alheid, et al., 1995). Based on cellular morphology and overall characteristics it can be stated that neurons of the cortical nucleus are very similar to their counterparts found in the BLA (McDonald, 1992; Alheid, et al., 1995). However, when making these judgements and classifications one must be careful not to base the function of a particular amygdaloid nuclear group on cellular morphology alone, since regions like the basolateral amygdaloid complex have been shown to have neural connections and biological functions which are unique and different to that of the cortical amygdaloid nucleus (Hitchcock and Davis, 1986a; 1986b; 1991; Campeau and Davis, 1995; McDonald, 1992; Pitkänen, 2000; Pitkänen, Steffanacci, Farb, Go, LeDoux, and Amaral, 1995; Blanchard and Blanchard, 1972; 1997; Kemble, Blanchard and Blanchard, 1998).

Pyramidal and Non-Pyramidal Neurons of the Nucleus of the Lateral Olfactory Tract

The nucleus of the lateral olfactory tract (NLOT) which is located in the very anterior portion of the superficial group contains medium-sized pyramidal neurons in the cell-dense layer II along with a mixed variety of large neurons in layer III that serve to encapsulate the nucleus (McDonald, 1992; Alheid, et al., 1995). Alheid, et al., 1995 notes that the pyramidal cells of layer II send thick densely spined apical dendrites all the way to the pial surface where upon entering the molecular layer they repeatedly divide to enhance innervation. In stark contrast, the basal dendrites, which are finer and shorter than their apical counterparts, generally remain confined to layer II and form numerous collaterals near the soma of the parent cell (Alheid, et al., 1995). Thus, the majority of the neurons in NLOT are spiny neurons that may exhibit pyramidal, semipyramidal, or stellate physical characteristics (McDonald, 1983b; McDonald, 1992; Alheid, 1995). The rest of the neurons make up a heterogeneous population of spine-sparse nonpyramidal cells that are similar to stellate cells (McDonald, 1983b; Millhouse and Uemura-Sumi, 1985; Alheid, et al., 1995).

Spiny pyramidal neurons are found primarily in layer II and are similar to the spiny pyramidal neurons in the cortical nucleus. As mentioned above, the basal dendrites of these neurons are short and tend to branch out near the soma of the parent cell whereas, the apical dendrites extend into layer I and send out numerous branch-like projections (McDonald, 1992; Alheid, et al., 1995). As a rule neurons in layer III are of the large spiny stellate variety whose axons emit collaterals that arborize mainly within layer III however, the main axons of these neurons travel dorsally with a handful observed entering the commissural bundle of the stria terminalis (McDonald, 1983b; McDonald, 1992).

Finally, all three layers of the NLOT contain a mixed variety of spine-sparse nonpyramidal neurons that display dense local axonal arborizations that are typically a common feature or characteristic of local circuit neurons (McDonald, 1983b; Millhouse and Uemura-Sumi, 1985; McDonald, 1992). These particular neurons are categorised as stellate or small non-spiny cell types and generally share much of the same physical characteristics as their counterparts found in the basolateral amygdaloid complex (Alheid, et al., 1995). Hence, these stellate and nonspiny neurons may be inhibitory neurons that

rely on GABAergic tone in order to limit excitatory potentials in the spine rich pyramidal neurons found in layer II of the NLOT.

3.13: The Centromedial Nuclear Group

In the rat, the centromedial group is situated in the dorsomedial portion of the amygdala and is made up of the central and medial nuclei. The remaining amygdaloid nuclei in this group include the anterior amygdaloid area, the AHA, and the intercalated nuclei (McDonald, 1998; Amaral, et al., 1992; Pitkänen, 2000). The BNST is also considered to be part of the centromedial nuclear group, but should receive special consideration when being categorized, since it is considered by some to be a rostral extension of both the central and medial amygdaloid nuclear regions (Alheid and Heimer, 1988; Johnston, 1923; Alheid, et al., 1995).

The central nucleus is located slightly dorsal and lateral to the medial nucleus and is bordered laterally by the amygdalostriatal transition area (AStr) and basolateral amygdaloid group. In contrast, the medial amygdaloid nucleus is located medial to the anterior basomedial and cortical amygdaloid nuclei and should not be confused with the medial subdivision of the central nucleus which lies dorsal to the basomedial and intercalated amygdaloid nuclei (McDonald, 1992; McDonald, 1998; Pitkänen, 2000; Paxinos and Watson, 1986). Thus, the medial nucleus can best be described as being situated in the dorsomedial corner of the temporal/piriform lobe in an area just lateral to the optic tract (McDonald, 1998). The anterior amygdaloid area is located rostral to the medial amygdaloid nucleus and mediodorsal to the nucleus of the lateral olfactory tract in the rat (Hall, 1972, McDonald, 1992; McDonald, 1998) and as such makes up part of the rostral pole of the amygdala (Alheid, et al., 1995; McDonald, 1998).

In contrast, the AHA is interposed between the hippocampal formation and the caudomedial portions of the amygdala (Price, et al., 1987; McDonald, 1998) and is found at more caudal levels as well. The intercalated amygdalar nuclei are made up of a narrow cluster of densely-packed neurons located medial to the basolateral amygdaloid complex, ventral to the central nucleus, and lateral to the medial amygdaloid nuclei, and are typically associated with the fibre bundles that separate the various amygdaloid nuclei (McDonald, 1998). Immunoreactive research experiments have demonstrated that the intercalated cell

group expresses high levels of tyrosine hydroxylase and dopamine β-hydroxylase immunoreactive fibres (Freeman and Cassell, 1994; Fuxe, Jacobsen, Höistad, Tinner, Jansson, Staines and Agnati, 2003) as well as sizeable populations of dopamine D_1 and D_2 receptors possibly located on GABAergic neurons (Scibilia, Lachowicz and Kilts, 1992; Maltais, Drolet, and Falardeau, 2000; Fuxe, et al., 2003). Moreover, high levels of GABA-like immunoreactivity have been shown to occur in cells of the intercalated region (Nitecka and Ben-Ari, 1987). These findings are particularly important since dopamine receptor and/or GABA receptor-mediated transmission may serve to influence the magnitude of fear or stress-induced autonomic and behavioural responses (Fuxe, et al., 2003).

Finally, BNST is considered to be the rostral extension of the central and medial amygdaloid nuclei, or what some researchers refer to as part of the "extended amygdala" (Alheid and Heimer, 1988; Johnston, 1923; Alheid, et al., 1995). One of the early pioneering comparative neuroanatomists to extensively examine the BNST was Johnston (1923). Johnston's research (1923) provided compelling neuroanatomical data demonstrating that both ontogenetically and phylogenetically, the BNST and the central and medial amygdaloid nuclei develop from similar primordial cellular elements and this finding is consistent with Golgi studies which confirm that the centromedial amygdala and BNST have nearly identical cell types (McDonald, 1983a; 1992; 1998). The BNST is located ventral to the central amygdaloid nucleus, lateral to the medial nucleus and just dorsal to the intercalated nuclei (McDonald, 1998; Alheid, et al., 1995; Cassell, et al., 1999; Pitkänen, 2000). At the very rostral pole of the extended amygdala, the BNST eventually merges with the caudal and medial regions of the nucleus accumbens (Alheid, et al., 1995).

Histochemical staining that incorporates immunohistochemical receptor analytic techniques has revealed that the rostral BNST is intimately connected with and related to the shell and core regions of the nucleus accumbens (Grove, 1988a, 1988b; Záborszky, et al., 1985; Heimer, de Olmos, Alheid, and Záborszky 1991a; Heimer, Zahm, Churchill, Kalivis and Wohltmann, 1991b; Alheid, Beltramino, Braun, Miselis, Francois and de Olmos 1994; Alheid, et al., 1995). This may be important as far as fear-motivated learning is concerned since the nucleus accumbens, extended amygdala, and areas of the basal forebrain already appear to play a key role in learning and memorizing behavioural tasks

that are either appetitive in nature or rely on internal motivational states and the integrity of dopamine systems (Everitt, Morris, O'Brien, and Robbins, 1991; Gallagher and Holland, 1992; Gallagher, Graham and Holland 1989; Kapp, Whalen, Supple and Pascoe, 1992; Cardinal, Parkinson, Hall, and Everitt, 2002; Salamone and Correa, 2002).

The central amygdaloid nucleus is divided into lateral and medial subdivisions (McDonald, 1998; Pitkänen, 2000). The lateral subdivision of the central nucleus has been further divided into two smaller distinct units, these are, the lateral subdivision proper and the lateral capsular subdivision (McDonald, 1982a; McDonald, 1992; McDonald, 1998; Pitkänen, 2000). The lateral subdivision proper is located immediately lateral to the medial subdivision of the central nucleus. On the other hand, the lateral capsular subdivision encapsulates the lateral half of the lateral subdivision proper and eventually merges with the laterally adjacent AStr which makes up part of the ventral caudate putamen (McDonald, 1982a; 1992). McDonald, (1982a) and Cassell, Gray and Kiss (1986) using immunohistochemical and selective staining techniques, also identified an intermediate and ventral subdivision located between the medial and lateral subdivision of the central amygdaloid nucleus.

Cell Type and Morphology

The neurons located in the medial subdivision of the central nucleus of the rat are generally small to medium sized ovoid or fusiform cell bodies that have three or four primary dendrites that exhibit minimal varicosity and have a low to moderate density of dendritic spines (McDonald, 1982a; Cassell and Gray, 1989; McDonald, 1992). The axons of these neurons emit numerous slender and finely beaded collaterals that send out only a few branches before exiting the nucleus through its dorsal or medial boundaries (McDonald, 1992). A small homogenous population of cells with practically no dendrites are also found in the medial subdivision of the central amygdaloid nucleus in the rat (McDonald, 1982a; Cassell and Gray 1989) and these are similar to the small to medium-sized pale cells with a low packing density observed in the medial subdivision of the monkey central amygdala (Amaral, et al., 1992). Thus, the cell types of the centromedial nuclear group do not resemble those of the cerebral cortex or those found in the basolateral and superficial cortex-like amygdaloid nuclear groups, rather they are more similar to the

medium-sized spiny neurons of the adjacent striatum (McDonald, 1998). The only exception to this rule may be the AHA which does have a significant population of cortex-like spiny pyramidal (type P cells) and spine-sparse stellate neurons (McDonald, 1992; 1998; Alheid, et al., 1995).

The principal cell type in the lateral subdivision of the central amygdaloid nucleus is also of the medium-sized spiny variety and these are found in abundance in both the lateral subdivision proper and the lateral capsular subdivision of the lateral division of the central nucleus (McDonald, 1982a; Cassell and Gray, 1989). As a rule, these cells are ovoid in shape and have several primary and secondary dendrites leaving the soma. Each of these dendrites forms robust dendritic arborization with up to as many as six branch points, some of which fan out only a short distance from the dendrite (McDonald, 1982a; 1992). This primary proximal branching is given off in such a quick succession that the cells tend to form a dendritic arborization that is tufted (i.e. feather-like) in appearance (McDonald, 1982a; 1992). In contrast, the more distal or secondary dendritic branches have an even higher density of dendritic spines, thus making the neurons in this region the most spine dense of any found in the entire amygdaloid complex (McDonald, 1982; 1992). The axons of these medium-sized spiny neurons also give off many beaded collaterals that branch out in the region of the parent cell. Many of these axonal collaterals are observed forming intimate connections with the dendrites of other medium-sized spiny neurons and on the dendrites of the parent cell (McDonald, 1982a; McDonald, 1992). In addition to the spiny neurons, the lateral regions of the rat central amygdala also contain a population of small to medium sized spine-sparse neurons. These spine-sparse cells send axons with several beaded collaterals that travel only a short distance from the parent cells and form contacts with neighbouring cells (McDonald, 1982; Cassell and Gray, 1989).

Several studies have demonstrated that the central amygdaloid nucleus contains dopamine D_1 and D_2 receptor subtypes as well as a substantial number of fibres that express high levels of immunoreactivity for dopamine metabolites (Meador-Woodruff, Mansour, Healy, Kuehn, Zhou, Bunzow, Huda, Civelli, and Watson, 1991; Meador-Woodruff, Mansour, Bunzow, Van Tol, Watson, and Civelli, 1989; Wamsley, Gehlert, Filloux and Dawson, 1989; Dawson, Barone, Sidhu, Wamsley and Chase, 1988; Freedman and Cassell, 1994; Scibilia, et al., 1992; Asan, 1997). Thus, dopamine-mediated processes in the central

nucleus of the amygdala may significantly influence the cell firing rates and the rate of LTP induction (see Rosenkranz and Grace, 1999; Bissière, Humeau and Lüthi, 2003). This point will take on greater significance when later Chapters of this thesis discuss the importance of amygdaloid dopamine receptors in conditioned fear learning and expression.

In addition to dopamine, the medium-sized spiny neurons found in the lateral portions of the central nucleus of the amygdala also display robust immunoreactivity for GABA and several neuropeptides such as somatostatin, neurotensin, corticotropin-releasing factor (CRF), cholecystokinin (CCK), and enkephalin (Sun and Cassell, 1993; Nitecka and Ben-Ari, 1987; Cassell, Gray, and Kiss, 1986; Cassell and Gray, 1989; McDonald, 1989). More specifically, Cassell and Gray, (1989) observed that somatostatin, neurotensin, enkephalin and CRF immunoreactivity occurred in the medium-sized spiny and larger pyramiform cells situated in the lateral aspects of the central amygdaloid nucleus. Finally, neuropeptide Y immunoreactivity axons were observed primarily in the medial aspects of the central nucleus of the amygdala however, a substantial population of neurons expressing immunoreactivity for neuropeptide Y also can be found in the medial amygdaloid nucleus and in the ventral aspects of the BNST (Gustafson, Card, and Moore, 1986).

Cell Types of the Medial Amygdaloid Nucleus

The medial nucleus, not to be confused with the medial subdivision of the central nucleus, only contains small to medium-sized cells that have two to four primary dendrites which give rise to a few branches. These dendritic branches usually have a moderate to sparse dendritic spine density and extend for considerable distances within the medial nucleus itself (McDonald, 1992; Yu, 1969 in McDonald, 1992; Millhouse and De Olmos, 1981). This neuronal organization and dendritic morphology appears to be relatively similar in the cat (Hall, 1972; Tömböl and Szafranska-Kosmal, 1972; Kamal and Tömböl, 1975), the dog (Mukina and Leontovich, 1970) and the rat (Yu, 1969; Millhouse and De Olmos, 1981). The only exception to the rule is that some neurons in the rat medial nucleus have thick dendrites that feature a very dense covering of spines, which is similar in many respects to the spine density of neurons observed in the lateral portion of the central nucleus (Millhouse and De Olmos, 1981; McDonald, 1992). The axons of medial amygdaloid neurons travel medially towards the basal nucleus or course dorsomedially

toward the stria terminalis (Tömböl and Szafranska-Kosmal, 1972; Kamal and Tömböl, 1975). Some of these axons emit a small number of sparsely branched collaterals that mainly limit themselves to the confines of the medial nucleus (Kamal and Tömböl, 1975), thus it appears that there are very few, if any local circuit neurons in the medial amygdaloid nucleus (McDonald, 1992).

Also, like the central nucleus, the medial nucleus of the amygdala exhibits high levels of immunoreactivity for GABA and neuropeptide Y (Nitecka and Ben-Ari, 1987; Gustafson, et al., 1986). In particular, caudal portions of the medial amygdaloid nucleus have been shown to contain high levels of GABA immunoreactivity (Nitecka and Ben-Ari, 1987). It is important to note that most of this GABA-like immunoreactivity in the medial nucleus of the amygdala was observed in an area close to the intercalated cell groups (Nitecka and Ben-Ari, 1987). With respect to neuropeptide Y, a large number of neurons in the medial amygdaloid nucleus express immunoreactivity for neuropeptide Y, with the largest concentration immunoreactive cells and axons found in the ventral aspects of the BNST and in the medial amygdaloid nucleus just ventrolateral to the optic tract in a coronal plane approximately -2.80 mm posterior to bregma in the rat (Gustafson, et al., 1986).

Cell Types of the BNST

The BNST is divided into a medial and lateral subdivision and the neurons which occupy these nuclei are virtually identical to their counterparts located in the medial and central amygdaloid nuclei respectively (McDonald, 1992; Alheid, et al., 1995; Cassell, et al., 1999). The lateral subdivision of the BNST contains medium-sized spiny neurons with a stellate dendritic configuration that is similar to cells found in the central amygdaloid nucleus and adjacent striatum (McDonald, 1992). The general physical shape and appearance of neurons in the medial subdivision of the BNST, including their dendritic spine density, collateral varicosities, and dendritic branching pattern is almost identical to cells observed in the medial amygdaloid nucleus (McDonald, 1992; Alheid, et al., 1995). Thus, Alheid and Heimer (1988) seem to be quite correct in their assertion that the BNST, substantia innominata, and portions of the centromedial amygdala form the extended amygdala (also see; Alheid, et al., 1995; Cassell, et al., 1999).

It is also important to note that the medial subdivision of the BNST is more heterogeneous as far as cell typology is concerned. As a result, the medial subdivision of the BNST has been further divided into anterior, ventral and posterior portions (Alheid, et al., 1995). In the medial aspects of the anterior portion of the medial subdivision of the BNST, cells are typically medium-sized, asymmetrically shaped and spread out amid the fibre bundles of the stria terminalis. In contrast, cells found in the lateral aspects of the anterior region of the medial BNST are smaller and densely packed (Alheid, et al., 1995). The ventral portion of the medial subdivision of the BNST is made up of primarily medium-sized cells with some larger randomly arranged cells that are darker staining than the closely packed neurons found in the lateral aspects of the medial BNST subdivision (Alheid, et al., 1995). The posterior region of the medial subdivision of the BNST is by far the largest and the longest subdivision and contains numerous cell types that have a column-like arrangement (Alheid, et al., 1995).

Due to the posterior region's heterogeneous cell population, the posterior subdivision of the medial subdivision of the BNST has been divided further into three separate cell columns that reflect the cellular diversity found in this particular area. In general, there is a medial small and dense-celled column that contains dark staining and oval shaped neurons which are found in the most medial portions of the posterior medial subdivision of the BNST. Next there is an intermediate medium-sized cellular column that is primarily made up of angular-shaped or spindle-like medium sized cells that stain lightly and are randomly dispersed throughout this region. Finally, there is a laterally located large-celled columnar group that is composed of loosely arranged neurons that are medium to large in size (Alheid, et al., 1995).

Most of the larger cells of this region are positioned in such a way that they run parallel to most of the incoming fibres of the stria terminalis (Alheid, et al., 1995) suggesting that these large neurons likely are involved in receiving and transmitting sensory information from various brain regions via the stria terminalis. This notion is supported by the work of Alheid, et al., (1995) who have observed that the dendrites of the large celled column are oriented in a transverse fashion to the incoming fibres of the stria terminalis and by research demonstrating that the BNST is involved in orchestrating anxiety-like behaviours in lab rats (e.g. potentiated acoustic startle responding) that have been exposed to bright

lighting conditions (see Walker and Davis, 1996; Davis, Walker and Lee, 1997; Walker and Davis, 1997a,b; Davis, 1998). For example, Walker and Davis (1997b) found that inactivation of the BNST impaired bright-light-enhanced startle responding, whereas inactivation of the central amygdala had no effect.

Cells Types of the Anterior Amygdaloid Area, Amygdalohippocampal Area and Intercalated Nuclei

Neurons of the anterior amygdaloid area are medium-sized and generally have round, ovoid, or triangular shaped cell bodies (McDonald, 1992; Alheid, et al., 1995). Golgi staining techniques indicate that these cells typically send out about three or four primary dendrites which branch sparingly and have a low to moderate density of dendritic spines (Hall, 1972; McDonald, 1992; Alheid, et al., 1995). An additional population of medium-sized neurons also exits in the anterior amygdaloid area but these contain beaded dendrites and very few dendritic spines (McDonald, 1992; Alheid, et al., 1995). Finally, there are also some larger stellate and fusiform neurons strewn amongst the other small and medium-sized cell types however, these larger neurons are found in the deeper recesses of the anterior amygdaloid area and generally bear a striking resemblance to their counterparts in the medial amygdaloid nucleus (McDonald, 1992; Alheid, et al., 1995).

Although McDonald (1992) suggests that the anterior amygdaloid area should be classified as part of the extended amygdala, others tend to disagree (see Alheid and Heimer, 1988; Alheid, et al., 1995) arguing that the anterior amygdaloid area, like the central or medial amygdaloid nucleus, sends numerous extrinsic projections that reach both the medial and lateral aspects of the hypothalamus and parts of the olfactory cortex. Given this impressive array of cortical and subcortical projections and its possible impact on basal forebrain and subcortical functioning as it relates to motivational learning directed by biologically significant cues (see Everitt, Morris, O'Brien, and Robbins, 1991; Gallagher and Holland, 1992; Gallagher, Graham and Holland 1989; Kapp, Whalen, Supple and Pascoe, 1992; Cardinal, et al., 2002; Salamone and Correa, 2002) it is essential that the anterior amygdaloid area (AAA) receives additional scientific examination to determine the role this nucleus plays in fear-motivated learning.

The AHA contains both cortex-like neurons including spiny pyramidal (type P cells) and spine-sparse stellate type S cells (McDonald, 1992; Alheid, et al., 1995; McDonald 1998). In general, cells of the AHA are slightly larger, more densely packed, and stain more deeply than cells found in any other neighbouring amygdaloid nuclei, thus making it easier to delineate the nuclear boundaries of this region (McDonald, 1992; Alheid, et al., 1995; McDonald, 1998). The anterior and lateral aspect of the AHA contains a population of neurons that are vaguely similar to neurons found in the posterior portions of the basolateral amygdaloid nucleus (Alheid, et al., 1995; Krettek and Price, 1978b).

However, as in the BLA, the cell types of the AHA do not consist of a continuous homogenous population of neurons, rather some variability and subtle diversity seems to exist. For example, the neurons of the anterolateral AHA are typically medium to large in size and are of the dark staining variety. In contrast, neurons found in the posterior and medial regions of the AHA are generally smaller and more densely packed with light staining qualities (Alheid, et al., 1995; Krettek and Price, 1978b; McDonald, 1998). These cytocarchitectural differences make it possible to divide the AHA into the deep lying anterolateral and the more superficial posteromedial subdivisions (Alheid, et al., 1995).

The neurons of the intercalated amygdaloid nuclei are small ovoid or elongated plate-shaped cells (7-12 μm; in diameter) with extremely spiny dendrites and their size and morphology makes them look like smaller versions of the medium-sized spiny neurons found in the striatum and lateral portions of the central amygdaloid nucleus (McDonald, 1992; Millhouse, 1986). The axons of these cells give off several short collaterals that branch off in the vicinity of the parent cell making it likely that these connections represent an inhibitory feedback loop (McDonald, 1992). However, at greater distances from the soma the axons of these cells send several collaterals into the basolateral and central amygdaloid nuclei (Tömböl and Szafranska-Kosmal, 1972; Millhouse, 1986) and immunohistochemical studies indicate that many of these projection neurons are GABAergic (Nitecka and Ben-Ari, 1987). These immunohistochemical and Golgi staining studies mentioned above are congruent with several retrograde tracing studies demonstrating that the intercalated cells send efferents to the central, medial, and basolateral amygdalar nuclear groups (Paŕe and Smith, 1993a; 1993b). Also, in addition to

the spiny neurons, there is a population of small spine-sparse neurons with highly arborized axons that are characteristic of local circuit neurons (Millhouse, 1986).

The last major cell type found in the intercalated nuclei of the rat are large neurons that are usually between 15-30 μm in length along the long axis with cell bodies being as large as 60 μm in diameter in the rat (Millhouse, 1986; Millhouse and De Olmos, 1983). These rather large neurons are subdivided into two classes based on their dendritic spine densities and they include the large spiny and large spine-sparse types that in many instances look similar to the large nonpyramidal neurons found in the basolateral amygdaloid complex (Millhouse, 1988; McDonald, 1992; Alheid, et al., 1995). Since these large neurons and their primary dendrites appear to be found in close proximity to boundary regions between immediately adjacent amygdaloid nuclei (Millhouse, 1986; Millhouse and De Olmos, 1983) these cells may either act as signal amplification and detection centres that facilitate communication between adjacent amygdaloid nuclei or they may act as a barrier to confine synaptic transmission within nuclear regions, thus preventing signal transmission from one nuclear region inadvertently flowing into another one. This is a particularly interesting supposition since the intercalated nuclei are located between the deep cortex-like nuclei of the basolateral complex laterally and the superficial cortex-like medial and cortical amygdaloid nuclei medially and ventrally. This view will assume a greater degree of significance following later discussions of the intrinsic connections of the amygdaloid nuclei. These will demonstrate that there is a unidirectional bias of efferent projections which channel information from the deep cortex-like amygdaloid nuclei to non-cortex-like amygdaloid nuclei.

3.14: Summary

In summary, most amygdaloid nuclei can be divided into two distinct groups on the basis of cytology and cellular morphology; these are the cortex-like and the noncortex-like amygdaloid nuclei. The cortex-like nuclei include the lateral, basal, and accessory basal (the deep amygdaloid nuclei) as well as the superficial amygdaloid nuclear group which includes the nucleus of the lateral olfactory tract, the periamygdaloid cortex, and the anterior and posterior cortical nuclei (Amaral, et al., 1992; McDonald, 1998). Included in the cortex-like cell group is the AHA because it too has been shown to contain spiny-

pyramidal neurons (McDonald, 1992; Alheid, et al., 1995). The principle neurons contained by these amygdaloid nuclei are the spiny pyramidal (or modified pyramidal) projection neurons which make up the majority of neurons residing in these regions and the spine-sparse nonpyramidal neurons that are fewer in number and function primarily as local circuit neurons that may be used to modulate amygdaloidal output via their intimate contacts with the pyramidal neurons (McDonald, 1992).

The noncortex-like nuclei include the central and medial amygdaloid nuclei, the BNST, the substantia innominata and intercalated nuclei (McDonald, 1992). In general, the principle neurons residing in the noncortex-like amygdaloid nuclei do not have a pyramidal-like structural configuration rather the cells are more stellate-like and have variable dendritic spine density (McDonald, 1992). The cytological differences between these two amygdaloid nuclear groups, in terms of cell type and axonal arborization, determines to a great extent what role is played by these neurons and their respective nuclei in managing the intrinsic circuitry and ultimate output of the amygdala as it communicates not only with itself (via intra-amygdaloid projections) but also with other cortical and subcortical brain nuclei (via amygdalopedal and amygdalofugal pathways).

3.2: Intrinsic Connections of the Amygdala

A wealth of neuroanatomical research indicates that the amygdala has a rich and complex internuclear connectivity (Krettek and Price, 1978b; Cassell, et al., 1999; McDonald, 1992; 1998; Doron and LeDoux, 1999; Pitkänen, 2000; Turner and Herkenham, 1991; Jolkkonen, Pikkarainen, Kemppainen and Pitkänen, 2001). The intrinsic connections between various amygdalaloidal nuclei are vast and quite intricate in most of the mammalian species investigated thus far, however, as a rule, the general organization of intraamygdalar connections seems to be similar in the rat, cat, and monkey (Price, Russchen and Amaral, 1987; Savander, Go, LeDoux, and Pitkänen, 1995; 1996a; Savander, Miettinen, LeDoux, and Pitkänen, 1997). This cross-species similarity is advantageous since it makes it possible to use mammals lower on the phylogenic scale to make predictions about how intra-amygdaloid circuits are organised in higher primates such as humans. This is especially important since most humans are reluctant to forfeit their amygdala for scientific experimentation and dissection.

The cellular configuration and cytoarchitecture of any particular amygdaloid nuclei to a large extent determines the degree of connectivity that it will have with other nuclear groups in the region. For example, the cortex-like amygdaloid nuclei have robust connections with each other and with noncortex-like nuclei. In stark contrast, the noncortex-like nuclei of the amygdala have relatively few reciprocal projections back to cortex-like nuclei (McDonald, 1992; Savander, et al., 1995; 1996a; Savander, et al., 1997; Pitkänen, 2000). Thus, the cortex-like nuclei which include the lateral, basal, accessory basal and the anterior and posterior cortical amygdaloid nuclei project heavily to the noncortex-like central and medial amygdaloid nuclei (Savander et al., 1995; 1996a; 1996b; Savander, et al., 1997). The noncortex-like central and medial nuclei, on the other hand, provide only a sparse projection back to the cortex-like nuclei (McDonald, 1992). As such, the flow of information or degree of connectivity from the cortex-like nuclei to non-cortex nuclei can be viewed as almost exclusively unidirectional and hierarchical in nature, however some researcher have found that the information flow within the amygdaloid complex is more reciprocal than first thought (see Pitkänen, Savander and LeDoux, 1997).

It is important to note that this type of unidirectional connective arrangement is also found, albeit to a lesser extent, between the cortex-like deep amygdaloid nuclei (i.e. lateral, basal, accessory basal nuclei) and the cortex-like superficial amygdaloid nuclei (i.e. the nucleus of the lateral olfactory tract, the periamygdaloid cortex and the anterior and posterior cortical nuclei). Thus, projections of the lateral nucleus to the accessory basal and periamygdaloid cortex are very robust as are those from the basal nucleus and accessory basal to the periamygdaloid cortex and anterior cortical nucleus (McDonald, 1992; Pitkänen, et al., 1995; Savander, et al., 1995; Savander, LeDoux, and Pitkänen, 1996a; 1996b). Conversely, the reciprocal projections from the cortex-like superficial amygdaloid nuclei back to the cortex-like deep amygdaloid nuclei are not nearly as strong, thus highlighting the almost hierarchical arrangement of intraamygdaloid neural connections and pathways.

Taking this connective arrangement into account, complex and highly processed polymodal information (i.e. auditory, visual, somatosensory, olfactory and nociceptive) from the prefrontal, perirhinal, entorhinal, and temporoinsular cortices along with sensory specific unimodal input from the thalamus and midbrain nuclei like the parabrachial

nucleus (Bernard, Peschanski and Besson, 1989) and ventral tegmental area (Swanson, 1982) converges primarily on the deep cortex-like nuclei found in the basolateral complex (McDonald, 1992; McDonald, 1998; Alheid, et al., 1995). Nociceptive information from the periaqueductal gray (PAG) also reaches these amygdaloid regions via the midline and intralaminar thalamic nuclei which receives a robust projection from cells located in the mediolateral portions of the PAG (Beitz, 1995; Behbehani, 1995). However, some of this sensory information may also target the cortex-like superficial amygdaloid nuclei and the noncortex-like nuclei such as the central nucleus. One such example is that of the parabrachial nucleus which relays nociceptive information directly to the central nucleus of the amygdala (Bernard, Peschanski and Besson, 1989; Bernard and Besson, 1990). Another example is the anterior olfactory nucleus which sends olfactory information to the nucleus of the lateral olfactory tract, the posterior cortical nucleus of the amygdala and the medial nucleus of the amygdala (Luskin and Price, 1983).

At the level of the lateral, basal and accessory basal nuclei which collectively make up the basolateral complex, sensory information from various cortical regions is exchanged via intrinsic connections between the lateral and accessory basal nucleus, and between the basal and accessory basal nucleus (McDonald, 1992; Price, et al., 1987; De Olmos, et al 1985; Savander, et al., 1995; 1996a; 1996b; Savander, et al., 1997). This sensory information is then transmitted from these deep cortex-like amygdaloid nuclei via intra-amygdalar neural pathways in one of two directions. Firstly, some cortical sensory input from the basolateral nuclei targets the superficial nuclei (i.e. the periamygdaloid cortex, anterior and posterior cortical nuclei, and nucleus of the lateral olfactory tract) which together form a region that is involved in the integration of olfactory information (McDonald, 1992; 1998; Luskin and Price, 1983; Pitkänen, et al., 1995). These superficial nuclei innervate the medial nucleus of the amygdala which in turn projects to the medial hypothalamus (Krettek and Price, 1978a,b; McDonald, 1992). Secondly, sensory information (i.e. mainly auditory, visual and somatosensory) from the basolateral amygdaloid complex reaches the noncortex-like central nucleus of the amygdala by way of the extensive and almost unidirectional projections that deep cortex-like nuclei share with noncortex-like nuclei (McDonald, 1992; Pitkänen, et al., 1995). The central nucleus of the amygdala in turn projects to the lateral hypothalamus and various nuclei in the midbrain

and pons region such as the substantia nigra, brachium conjunctivum, central tegmental field, ventral tegmental area, dorsal motor nucleus of the vagus, periaqueductal gray, retrorubral field, parabrachial nucleus, and the reticularis pontis caudalis to mention a few (Krettek and Price, 1978a; Hopkins and Holstege, 1978; Rosen, Hitchcock, Sananes, Miserendino, and Davis, 1991; Wallace, Magnuson and Gray, 1989; Post and Mai, 1980; Swanson, 1982; Price, Slotnick and Revial, 1991).

Thus, the central nucleus acts as a major output nucleus of the amygdala in that it first collects highly processed and emotionally charged information from a variety of amygdaloid nuclei and then passes this information on to discrete hypothalamic and midbrain nuclei that mediate selective autonomic and behavioural responses that correspond to the specific emotion in question. It is therefore plausible that intrinsic amygdaloid connections and neurochemical processes that enhance synaptic transmission and help relay information both within the amygdala and between the amygdala and various cortical and subcortical regions may be of critical importance in understanding how conditioned fear is acquired, stored, retrieved, and expressed behaviourally (see Davis, 1992a,b,c; LeDoux, 1993; Pitkänen, et al., 1997; Fendt and Fanselow, 1999; Maren, 1996; 1999).

Anatomical and electrophysiological studies demonstrate that the lateral amygdaloid nucleus projects extensively to the basal nucleus and AStr but surprisingly projects very little to either the central or medial amygdaloid nuclear groups (Sefanacci, Farb, Pitkänen, Go, LeDoux, and Amaral, 1992; Jolkkonen, et al., 2001; Pitkänen et al., 1995; Savander, et al., 1997; Wang, Kang-Park, Wilson, and Moore, 2002). This indicates that various forms of sensory information in the lateral amygdaloid nucleus are first transmitted to the basal, accessory basal and AStr for processing before finally being sent to the central nucleus of the amygdala (Price et al., 1987; Pitkänen, et al., 1995; Savander, Go, LeDoux, and Pitkänen, 1996) and physiological evidence demonstrates that signal propagation through the lateral amygdala-amygdalostriatal pathway (La-AStr) is significantly faster and has a higher rate of transmission efficiency than signal transmission through the lateral amygdala-basolateral nuclear pathway (La-BL) (Wang, et al., 2002). For example, the rate of signal propagation decay (i.e. a measurement of the length constant) measured in the La-BL pathway was found to be significantly greater than measures obtained from the La-AStr

pathway (Wang, et al., 2002). Also, studies investigating the velocity of signal propagation (i.e. a result obtained by measuring the latency to peak response plotted against the distance travelled by a given impulse), found that impulses travelling through the La-BL pathway generally exhibit a significant decrease in propagation velocity, whereas similar impulses travelling through the La-AStr pathway actually display a marked acceleration of propagation velocity (Wang, et al., 2002). These physiological results not only indicate that signals travelling through the La-BL are slower but they also demonstrate just how the remarkable variability of intrinsic amygdaloid connectivity influences the efficacy of synaptic transmission processes.

Several lines of electrophysiological and pharmacological research seem to suggest that much of the variability that affects synaptic transmission is likely due to the pharmacological properties of neurons residing in the lateral, basolateral, and amygdalostriatal transition nuclear regions (Wang, et al., 2002; Wang, Wilson and Moore, 2001; Rainnie, Asprodini and Shinnick-Gallagher, 1991a; Rainnie, Asprodini and Shinnick-Gallagher, 1991b; Mahanty and Sah, 1998; Farb, Aoki, Milner, Kaneko and LeDoux, 1992; Nose, Higashi, Inokuchi and Hishi, 1991). For example, research results obtained from the Wang, et al., (2002) study indicate that the sensitivities to bicuculline (i.e. a $GABA_A$ receptor antagonist) and D-2-amino-5-phosphonovaleric acid (D-AP5; a NMDA receptor antagonist) are typically much higher in the La-BL pathway than in the La-AStr pathway since microinfusion with 20 μM of bicuculline significantly enhanced evoked responses in the basolateral nucleus (BL) when compared to the amygdalostriatal transition area (AStr), while a 50 μM application of D-AP5 significantly decreased evoked potentials to a far greater extent in the BL than in the AStr (Wang, et al., 2002). Furthermore, application of 20 μM of the AMPA receptor antagonist DNQX during paired-pulse inhibition testing not only produced stronger inhibition during the late phase of the signal in the BL than in the AStr but was also able to block signal propagation to both the BL and AStr when the lateral amygdaloid nucleus was stimulated (Wang, et al., 2002). These results indicate a role for GABA, NMDA, and AMPA receptor-mediated effects on synaptic transmission in the La-BL pathway.

Taken together, the reported electrophysiological and pharmacological characteristics and differences of these two intrinsic amygdalar pathways (i.e. La-BL versus La-AStr)

indicates that they may play slightly different, albeit complementary roles during fear conditioning and fear expression. For example, it has been hypothesised that the La-AStr pathway may be involved in the rapid and reflexive responses that occur during fear conditioning (i.e. jumping in response to light + footshock presentations) and fear expression (i.e. potentiated startle) while the La-BL pathway maybe involved in the enhancement and amplification of signal integration that may be necessary to facilitate amygdaloid LTP, fear learning and expression, and even various forms of instrumental behaviour that are biologically adaptive (Wang, et al., 2002; Stutzmann and LeDoux, 1999; Killcross, Robbins and Everitt, 1997; Rogan, Stäubli and LeDoux, 1997a; Walker and Davis, 2002; Liang, Hon and Davis, 1994). However, since the amygdala receives input from various cortical and subcortical brain regions, it is likely that intrinsic connectivity and physiochemical properties of the amygdala are not the only factors that contribute to sensory processing and fear learning. It is therefore important to discuss some of the cortical and subcortical projections to the amygdala because the sensory information supplied by these regions form the essential sensory elements that make associative fear learning and memory formation possible.

3.3: Cortical and Subcortical Sensory Projections to the Amygdala

The amygdaloid complex receives enormous volumes of sensory input and highly processed sensory information from a variety of cortical and subcortical regions in the brain (for a review see; McDonald, 1992; 1998; Amaral, et al., 1992; Pitkänen, 2000; also see; Shi and Davis, 1997; 1998; Fendt and Fanselow, 1999; Uwano, Nishijo, Ono, and Tamura, 1995; Ono, Nishijo and Uwano, 1995). Some of this sensory information reaches the amygdala by way of the thalamus or via midbrain nuclei that project directly to the amygdala (Ottersen and Ben-Ari, 1979; Russchen, 1982b; LeDoux, Ruggierro and Reis, 1985; LeDoux, Farb and Ruggierro, 1990a; Bernard, Alden and Besson, 1993; Ottersen, 1981; Swanson, 1982; Bordi and LeDoux, 1994; Turner and Herkenham, 1991). The amygdala also sends reciprocal projections back to many of these regions (Krettek and Price, 1978a; Amaral, et al., 1992), thus enabling the amygdala to continuously monitor and gate the level of sensory input that it receives. Other sensory information (i.e. the more complex variety) takes a more indirect route by first entering the thalamus and then being

passed on to the appropriate primary sensory cortices. The information is then passed through a series of cortical cascades where various cortical association areas add, refine, and process the information before finally sending it to the amygdala (McDonald, 1998; Amaral, et al., 1992).

In all animal species studied thus far, the cortical projections to the amygdala enter the subcortical white matter near the external capsule and then proceed directly to the amygdala primarily targeting the lateral, basal and central amygdaloid nuclei (Whitlock and Nauta, 1956; Druga, 1969; Lescault, 1971; Mascagni, McDonald and Coleman, 1993; Amaral, et al., 1992; McDonald, 1998). It is important to note that many midbrain and thalamic nuclei that deal with modality specific input from a variety of sources generally send efferents to many of the same amygdaloid nuclei that are targeted by cortical areas (Turner and Herkenham, 1991; Doran and LeDoux, 1999; McDonald, 1992; 1998). The amygdala keeps itself in the communication loop by sending projections back to many of the cortical and subcortical regions that supply it with either highly processed information or sensory specific input. In this sense the amygdala can be described as a strategically positioned and critical neural substrate that actively ties together cortical areas that process complex sensory information with regions involved in eliciting conditioned and emotionally charged autonomic, behavioural and motivational responses. In doing so, the amygdala may be one of the key brain regions that contribute to the subjective feelings and memories associated with fear. In order to garner a greater understanding and appreciation for the complexity of the amygdala's role in fear learning and emotional expression, it is first necessary to provide a brief description of the amygdala's connectivity with cortical and subcortical structures across a range of sensory modalities.

3.31: Auditory Cortical Projections to the Amygdala

The way in which cortically processed auditory information arrives at the cortex and ultimately reaches the amygdala is complex but can be easily understood since it primarily involves two separate auditory pathways. These two pathways are the lemniscal auditory pathway and the extralemniscal auditory pathway. Auditory information travelling via the lemniscal auditory pathway converges on the ventral division of the medial geniculate nucleus of the thalamus (vdMGN) whilst, the extralemniscal auditory pathway targets the

medial division of the medial geniculate nucleus of the thalamus (mdMGN) and the posterior intralaminar nucleus of the thalamus (PIN) (Iversen, Kupfermann, and Kandel, 2000; Turner, 1981; Turner and Herkenham, 1991; Ono, et al., 1995; Uwano, et al., 1995). It is at the level of the thalamus that the lemniscal and extralemniscal pathways functionally diverge to route auditory information in different directions. For example, the vdMGN, which is a continuation of the auditory lemniscal pathway, sends a projection only to the primary auditory cortex (Te1) and consequently does not directly project to the amygdala (Iversen, et al., 2000; Turner and Herkenham, 1991; LeDoux, 1993). Te1 in turn sends a series of cortical cascades that reach the auditory association areas (Te3, Te3R, Te2 and Te2D) and the polymodal associations areas such as the perirhinal and entorhinal cortices (Iversen, Kupfermann, and Kandel, 2000; LeDoux, Farb and Romanski, 1991; Turner and Zimmer, 1984; Mascagni, et al., 1993). These association areas then send the highly processed auditory information on to the lateral and basal amygdaloid nuclei (McDonald, 1998; LeDoux, Farb and Ruggiero, 1990; LeDoux, Farb and Romanski, 1991; Ono, et al., 1995; Uwano, et al., 1995). Additionally, the polymodal association areas (i.e. perirhinal and entorhinal cortices) also have the option of sending highly processed auditory information to the hippocampus and subiculum which in turn project to the lateral and accessory basal nuclei of the amygdala (LeDoux, 1993). In this way areas like the hippocampus and subiculum can add contextual richness to auditory information being sent to the amygdala.

In contrast, the mdMGN and PIN which receive input from the extralemniscal pathway send auditory information to the primary auditory area, the auditory association areas and to the lateral and basal amygdaloid nuclei (LeDoux, et al., 1990; Romanski and LeDoux, 1993; Romanski, LeDoux, Clugnet and Bordi, 1993; Iversen, Kupfermann, and Kandel, 2000). The extralemniscal auditory pathway can best be described as a divergent sensory pathway in that mdMGN and PIN send some auditory information directly to the lateral and basolateral amygdaloid nuclei while other auditory input is channelled to the primary auditory area Te1. Te1 then transmits this auditory information to Te2, the auditory association areas (Te3, Te3R, and Te2D) and to the perirhinal and entorhinal cortices in order to commence the cortical auditory cascade to the lateral and basolateral portions of the amygdala.

Thus, the mdMGN and PIN auditory projection to the amygdala, via the extralemniscal pathway, represents the direct thalamo-amygdaloid pathway whereas, the mvMGN lemniscal and part of the mdMGN/PIN extralemniscal pathways represent the more indirect thalamo-cortico-amygdaloid auditory pathways (Iversen, Kupfermann, and Kandel, 2000; Romanski and LeDoux, 1993; Romanski, LeDoux, Clugnet and Bordi, 1993; LeDoux. 1993) and a plethora of research indicates that pathways from the thalamus to the amygdala play a crucial role in fear learning (Romanski and LeDoux, 1992; Quirk, Repa and LeDoux, 1995; Quirk, Armony and LeDoux, 1997). Lesion and anatomical studies carried out by Romanski and LeDoux (1992; 1993) provide strong evidence that both thalamo-amygdalar and thalamo-cortico-amygdalar pathways provide auditory information to the amygdala and are involved in fear learning as measured by defensive freezing and FPS (Davis, 1992; 2000; Fendt and Fanselow, 1999; LeDoux, 1992; 2000; Romanski and LeDoux, 1992; 1993; Iwata, LeDoux, Meeley, Arneric and Reis, 1986).

In general, anatomical and electrophysiological evidence indicates that the rat amygdala receives cortically processed auditory information from the auditory association areas Te3, Te3R, Te2 and Te2D, along with a small discrete projection emanating from the ventral portions of the primary auditory cortex (Te1) (Romanski and LeDoux, 1993; Li, Stutzmann and LeDoux, 1997; LeDoux, Farb, and Romanski, 1991; Mascagni, et al., 1993; Shi and Cassell, 1997a; McDonald and Mascagni, 1996). Results obtained from anterograde and retrograde tracing studies indicate that neurons in the ventral section of the primary auditory cortex (Te1) innervate the dorsolateral subdivision of the lateral amygdaloid nucleus (LeDoux, Farb, and Romanski, 1991; Romanski and LeDoux, 1993) and provide auditory input to the amygdala. It is important to point out that the findings of LeDoux and colleagues (1991; 1993) mentioned above may represent a very small and discrete auditory projection from Te1 to the amygdala since several more recent anterograde studies failed to find a direct projection from Te1 to the amygdala (Mascagni et al., 1993; Shi and Cassell, 1997a). The anterograde study results obtained from both Mascagni, et al., (1993) and Shi and Cassell (1997a) indicate that Te1 sends robust cortical projections to the auditory association area (Te3) which in turn projects to the amygdala. For example, microinfusion of anterograde tracers into the auditory association area Te3 produced extensively labelled axonal fibres primarily in the dorsolateral subdivision of the lateral amygdaloid nucleus, the

lateral capsular division of the central nucleus and the AStr (Mascagni, et al., 1993 Romanski and LeDoux, 1993; Shi and Cassell, 1997a) with some sparse labelling reported in the ventromedial and ventrolateral subdivisions of the lateral nucleus (Shi and Cassell, 1997a).

Furthermore, the auditory association areas Te2, Te2D, Te3, and Te3R which together make up the specific and non-specific belt cortices receive input from auditory areas located in the medial geniculate nucleus of the thalamus and from Te1 (Scheel, 1988; LeDoux, Ruggiero and Reis, 1985). Auditory information from these association areas in turn targets specific amygdaloid nuclei and these projections appear to have some topographic arrangement. For example, anterograde tracer chemicals infused into Te2D generate labelled axon fibres and cell bodies in the lateral nucleus, lateral capsular subdivision of the central nucleus, and the basal nucleus (McDonald and Mascagni, 1996) whereas, similar infusions into Te3R produced only light labelling in the dorsolateral subdivision of the lateral nucleus (Mascagni, et al., 1993).

The results obtained from the anterograde studies mentioned above appear to be in general agreement with retrograde and electrophysiological studies in terms of how cortically processed auditory information reaches the amygdala (Ono, et al., 1995; Uwano, et al., 1995; LeDoux, et al., 1991; Mascagni, et al., 1993; LeDoux, et al., 1991). Thus, infusion of retrograde labelling materials into the lateral amygdaloid nucleus and portions of the magnocellular subdivision of the basal nucleus has been shown to produce labelled cells in either layers II and V of Te3 (LeDoux, et al., 1991) or very discrete portions of Te3 (Mascagni, et al., 1993). In addition, electrophysiological studies that examined the response latency of lateral amygdala neurons to auditory stimuli previously paired with footshock demonstrated that slower short response latencies (between 18-25 milliseconds) represent auditory information being sent via the longer indirect route involving the auditory association areas or belt cortices that make up part of the thalamo-cortico-amygdalar lemniscal and extralemniscal auditory pathways mentioned earlier (Romanski and LeDoux, 1993; Li, et al., 1997; Iversen, Kupfermann, and Kandel, 2000; Romanski and LeDoux, 1993; Romanski, LeDoux, Clugnet and Bordi, 1993). In contrast, the faster short latency responses (10-15ms) to excitatory auditory stimuli recorded from neurons in the dorsal and ventral subdivisions of the lateral nucleus represent the involvement of direct

auditory input from the medial geniculate and posterior intralaminar nuclei of the thalamus (Romanski and LeDoux, 1993; Li, et al., 1997). This direct pathway corresponds to the extralemniscal thalamo-amygdalar auditory pathway discussed earlier. Thus, in addition to receiving more highly processed cortical auditory information, the amygdala is also the recipient of direct and subcortically processed auditory input emanating from the acoustic thalamus (LeDoux, et al., 1990; Li, et al., 1997).

3.32: Auditory Subcortical Projections to the Amygdala

Neuroanatomical tracing studies and electrophysiological experiments indicate that the amygdala receives a great deal of subcortically processed auditory information from both the medial division of the medial geniculate nucleus of the thalamus (mdMGN) and the posterior intralaminar nucleus of the thalamus (PIN) (LeDoux, Ruggiero, and Reis, 1985; LeDoux, Farb, and Ruggiero, 1990; Romanski and LeDoux, 1992; LeDoux, Farb and Romanski, 1991; Turner and Herkenham, 1991; Romanski, Clugnet, Bordi and LeDoux, 1993; Bordi, LeDoux, Clugnet and Pavlides, 1993; Ono, et al., 1995; Uwano, Nishijo, Ono and Tamura, 1995). The mdMGN and the PIN form part of the direct extralemniscal thalamo-amygdalar auditory pathway that has been shown to undergo or be involved in long-term changes in synaptic plasticity following either tentanic stimulation (Clugnet and LeDoux, 1990; Rogan and LeDoux, 1995) or Pavlovian fear conditioning (Rogan, Stäubuli and LeDoux, 1995; Quirk, Repa and LeDoux, 1995; Quirk, Armony and LeDoux, 1997), and anterograde tracing studies demonstrate that the mdMGN and PIN both send efferents to the lateral amygdaloid nucleus (LeDoux, Farb and Ruggiero, 1990). Auditory information from these thalamic nuclei terminates almost exclusively in the dorsal and ventral subdivision of the lateral amygdaloid nucleus, however, some of this input reaches the basal nucleus as well (LeDoux, Farb, and Ruggiero, 1990; Romanski and LeDoux, 1992; Ono, et al., 1995; Uwano, et al., 1995; McDonald, 1998; LeDoux, 1993), thus, making the basolateral amygdaloid complex an area that is well suited to the processing of biologically relevant auditory stimuli (LeDoux, 1993; Ono, et al., 1995; Uwano, et al., 1995; LeDoux, 1993; Koch, 1999; Fendt and Fanselow, 1999) [see Figure 1 depicting how auditory information reaches the amygdala].

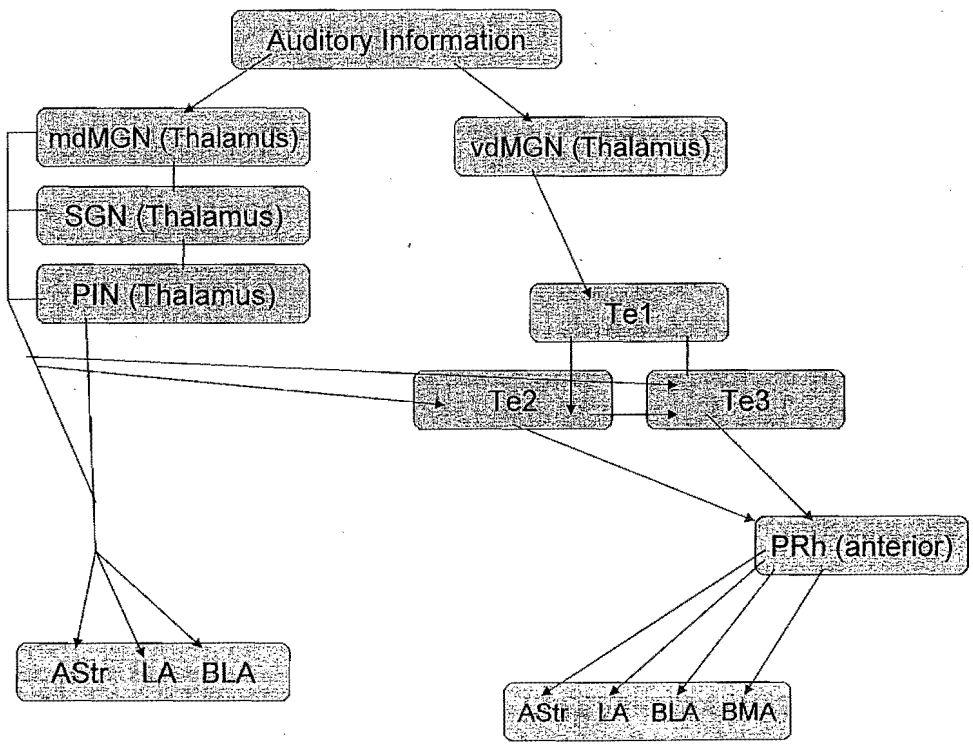

Figure 1 above depicts how auditory information reaches the amygdala. Note that auditory input is transmitted to the amygdala either via the sub-cortical and direct thalamo-amygdaloid route (left portion of Figure 1.) or via the longer and indirect cortical route that involves the thalamo-cortico-amygdaloid auditory pathways (right portion of Figure 1). The direct route involves the medial division of the medial geniculate nucleus (mdMGN), the supragenicuiate nucleus (SGN) and the posterior intralaminar nucleus (PIN) sending auditory information to the lateral amygdaloid nucleus (LA), amygdalostriatal transition area (AStr), and the basolateral amygdala (BLA). However, some auditory information is also sent to Te2 and Te3 before being transmitted to the perirhinal cortex (PRh) and on to the amygdala. The indirect cortical route begins in the ventral subdivision of the medial geniculate nucleus of the thalamus (vdMGN). From the vdMGN, auditory information is first transferred to Te1 and then on to Te2 and Te3 of the temporal cortex. Auditory signals are then sent to the anterior PRh before finally being transmitted to the AStr, LA, BLA, and basomedial amygdaloid nucleus (BMA).

3.33: Visual Cortical and Subcortical Projections to the Amygdala

Although a lot of information is available detailing the auditory and visual cascades to the amygdala, less is generally known about how visual information reaches the amygdala and whether thalamic inputs to the amygdala are more important for fear learning than are cortical projections to the amygdala. This has generated some interesting debates among researchers who study the neural substrates associated with fear learning when visual and/or auditory stimuli are used during fear-training (compare the views of Campeau and Davis, 1995; Rosen, et al., 1992; Davis, 1992; to those of Romanski and LeDoux, 1992; LeDoux, 1993; 2000; Corodimas and LeDoux, 1995). Nevertheless, on the basis of research conducted on the rat and monkey, it has generally been accepted that thalamo-amygdalar and thalamo-cortico-amygdalar visual pathways are involved in relaying visual information to the amygdala (McDonald, 1998; Vaudano, Legg, and Glickstein, 1990; Turner, Mishkin and Knapp, 1980; Turner and Herkenham, 1991; Webster, Ungerleider, and Bachevalier, 1991; Mascagni, et al., 1993; Romanski and LeDoux, 1993; Shi and Cassell, 1997a; Iwai and Yukie, 1987; Ono, et al., 1995; Shi and Davis, 2001). According to this research, visual information could reach the amygdala in a variety of ways, but the two most common paths include either a direct route via the lateral geniculate and lateral posterior nuclei of the thalamus to the amygdala or a more indirect means which includes the primary visual area passing information to visual association areas and other cortical regions (such as the perirhinal and temporal belt cortices) that innervate the amygdala (Mascagni, et al., 1993; Romanski and LeDoux, 1993; Shi and Cassell, 1997a; Iwai and Yukie, 1987; Webster, Ungerleider, and Bachevalier, 1991; LeDoux, 1993). For example, experiments incorporating autoradiographic and anterograde horse radish peroxidase (HRP) techniques carried out on the brains of several primate species found that rostral regions of the inferior temporal cortex innervate dorsal aspects of both the lateral amygdaloid nucleus and magnocellular basal nucleus (Van Hoesen, 1981; Webster, et al., 1991; Iwai and Yukie, 1987; Herzog and Van Hoesen, 1976).

Similarly, anatomical tracing studies and electrophysiological research on rats indicates that subcortical visual information arising from the lateral geniculate nucleus of the thalamus, the suprageniculate nucleus and zona incerta is channelled through primary and secondary visual areas to arrive at Te2 and the PRh. This neocortically processed visual

information from Te2 and the PRh is then forwarded to the AStr, and the lateral, basolateral and central amygdaloid nuclei (LeDoux, et al., 1991; Mascagni, et al., 1993; Turner and Zimmer, 1984; McDonald and Jackson, 1987; Turner and Herkenham, 1991; Ono, et al., 1995; Shi and Davis, 2001). Conversely, in the more direct subcortical thalamo-amygdalar visual information pathway, visual input from the lateral posterior nucleus of the thalamus is transferred directly to the AStr and the lateral and basolateral amygdaloid nuclei (LeDoux, et al., 1990) however, some of the visual input branches off and is consequently sent to Te2 and the PRh before arriving at the amygdala (LeDoux, et al., 1990; Ono, et al., 1995). Thus, there appears to be a high degree of overlap and convergence of cortical and subcortical processed visual information in the AStr and in the lateral, basolateral and central amygdaloid nuclei (see Figure 2).

The anatomical results discussed above are very compatible with much of the electrophysiological research carried out on visually responsive amygdaloid neurons of monkeys (Ono and Nishijo, 1992; Nakamura, Mikami and Kubota, 1992) and rats (Ono, et al., 1995; Uwano, et al., 1995). For example, most visually responsive neurons were reportedly found in the dorsal sections of the lateral, basolateral, central and basal magnocellular amygdaloid nuclei (Ono and Nishijo, 1992; Nakamura, Mikami and Kubota, 1992; Ono, et al., 1995; Uwano, et al., 1995) which corresponds well with the anatomical data obtained from axonal degeneration, HRP, and autoradiographic experimentation (Whitlock and Nauta, 1956; Jones and Powel, 1970; Van Hoesen, 1976; Turner, et al., 1980; Van Hoesen, 1981; Iwai and Yukie, 1987; Webster, et al., 1991). Additionally, since many of these amygdaloid neurons displayed activation in response to biologically relevant visual stimuli that was associated with either a reward or punishment (Ono and Nishijo, 1992; Ono, et al., 1995; Uwano, et al., 1995), they may make up a crucial part of the neurocircuitry that is responsible for the associative learning that occurs during Pavlovian fear conditioning.

Generally speaking, the lateral amygdaloid nucleus in the rat does not receive direct innervation from the primary visual cortex (Oc1), which is consistent with the anatomical evidence obtained from the monkey where the primary visual area of the occipital lobe (V1) does not project directly to the amygdala (Aggleton, Burton and Passingham, 1980; Turner, et al., 1980; Iwai and Yukie, 1987). However, in the rat Oc1 projects to Oc2

(secondary visual area) and to caudal portions of the PRh and since the PRh innervates the lateral amygdaloid nucleus (Ottersen, 1982), the PRh may serve as a conduit whereby visual information from Oc1 is transmitted to the amygdala (McDonald, 1998). For example, in addition to auditory responsive cells, area Te3 also contains numerous visually responsive neurons that target the dorsal PRh and have been shown to play an important role in visual recognition memory (Zhu, Brown and Aggleton, 1995a; 1995b; Mascagni, et al., 1993; LeDoux, et al., 1991; Quirk, Armony and LeDoux, 1997). Since the dorsal PRh projects to the lateral and basal amygdaloid nuclei (Shi and Cassell, 1997b) the finding of Zhu, et al., (1995a,b) provides us with some insight into how information about visual stimuli is processed and stored in the brain during classical fear conditioning.

In addition, Oc2 projects to Te2 (Miller and Vogt, 1984; McDonald and Mascagni, 1996; Shi and Cassell, 1997a) and PHA-L anterograde tracing studies have demonstrated that the amygdala receives input from a small group of visually responsive neurons in Oc2 that were located near the dorsal portion of Te2 (McDonald and Mascagni, 1996; Paxinos and Watson, 1986). Te2 receives visual information from the lateral posterior nucleus of the thalamus (Vaudana, Legg and Glickstein, 1990) and Oc2 (Miller and Vogt, 1984; McDonald and Mascagni, 1996; Shi and Cassell, 1997a; Vaudana, et al., 1990; Shi and Davis, 2001) and this may be of some importance as far as fear conditioning involving the amygdala is concerned, since Te2 neurons have been shown to play a crucial role in discriminating complex visual stimuli (Kolb, 1990). The Te2 area also receives innervation from cell groups located in Te3 and various other auditory belt cortices (Mascagni, et al., 1993; Shi and Cassell, 1997a) and may initiate processes designed to integrate the auditory and visual sensory modalities.

Chemical manipulation of the Te2 region with anterograde tracers has been shown to produce heavily labelled fibres in the basolateral nucleus, the lateral capsular subdivision of the central amygdaloid nucleus and the dorsal regions of the lateral nucleus with the most pronounced labelling terminating in the ventrolateral portions of the lateral amygdaloid nucleus and in the dorsomedial portions of the magnocellular division of the basal nucleus (Mascagni, et al., 1993; Shi and Cassell, 1997a). Finally, Te2 projects to the PRh, an area which may be involved in transmitting visual and polymodal information to the amygdaloid complex (Decon, Eichenbaum, Rosenberg, and Eckman, 1983; Miller and Vogt, 1984).

Thus, Te2 may directly or indirectly (via the PRh) pass on visual, auditory, somatosensory, or even nociceptive information to the amygdala. This polysensory information may converge in the amygdala and serve to facilitate associative learning process during Pavlovian fear conditioning especially when several sensory modalities are incorporated in the training procedures (see Ono, et al., 1995; Uwano, et al., 1995; LeDoux, 1993; Campeau and Davis, 1995a; Fendt and Fanselow, 1999; Koch, 1999; Shi and Davis, 2001).

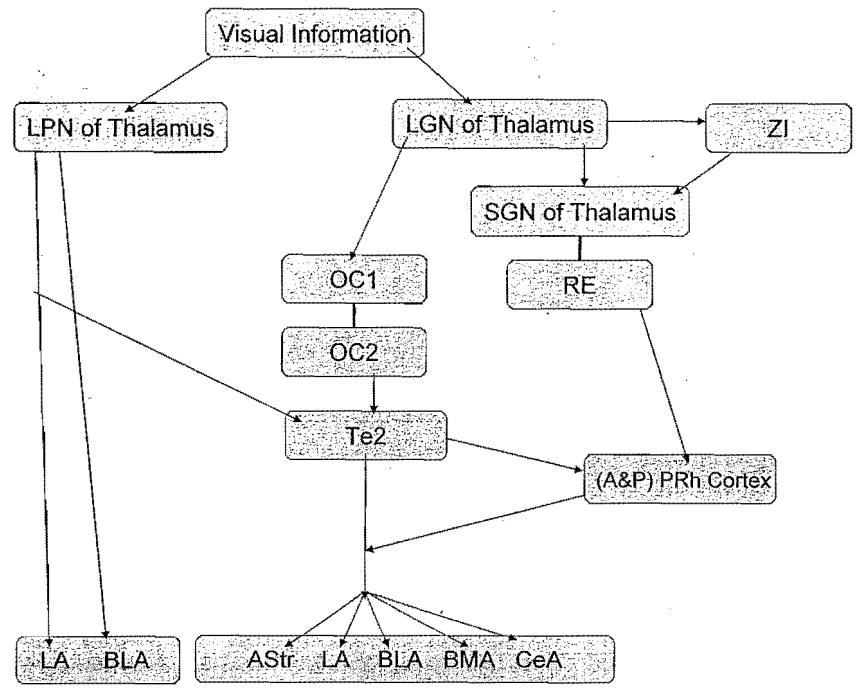

Figure 2 above provides a schematic description of how visual information reaches the amygdala. Visual sensory input reaches the amygdala through either direct thalamo-amygdaloid pathways (left portion of Figure 2) or via indirect thalamo-cortico-amygdaloid cascades (middle to right portion of Figure 2). The lateral posterior nucleus of the thalamus (LPN) sends visual information directly to the LA and BLA, but some visual information is transmitted to Te2 before reaching some amygdala regions (e.g. AStr, LA and BLA). The thalamo-cortico-amygdaloid visual cascade begins with the lateral geniculate nucleus of the thalamus (LGN) transmitting visio-sensory information to Oc1 (primary occipital cortex), Zona Incerta (ZI) and the suprageniculate nucleus of the thalamus (SGN). Oc1 sends the visual information to the secondary occipital cortex (Oc2). Oc2 in turn passes the visual input on to Te2. Te2 then transmits the visual information either directly to several amygdaloid nuclear regions (i.e. AStr, LA, BLA) or to the anterior and posterior portions of the perirhinal cortex (PRh). Visual information from the SGN also eventually reaches the PRh via the Reuniens Nucleus (RE). The visual information in the PRh is then sent on to various amygdaloid nuclei (e.g. AStr, LA, BLA, BMA, CeA) to complete the visual cascade. Note CeA = central nucleus of the amygdala, LA = lateral nucleus of the amygdala, AStr = amygdalostriatal transition area, BLA = basolateral amygdala, BMA = basomedial amygdaloid nucleus. .

3.4: Somatosensory, Visceral, and Gustatory Cortical and Subcortical Input to the Amygdala

3.41: Somatosensory Input to the Amygdala

In general detailed neuroanatomical information relating to the cortical somatosensory cascade to the rat amygdala is scant however, some research efforts employing biocytin (Shi, 1995 in McDonald, 1998) and PHA-L (McDonald and Mascagni, 1998 in McDonald, 1998) as anterograde tracers have provided some much needed information in this regard. The limited scope of information related to the rat will not present much of a problem since any additional information obtained from anatomical and electrophysiological studies carried out on the cat, and monkey will be incorporated into these discussions. Furthermore, some exhaustive electrophysiological research examining the responsiveness of amygdaloid neurons to a variety of sensory stimuli have been carried out on the rat (Ono, et al., 1995; Uwano, et al., 1995). Since these electrophysiological studies examine somatosensory and visceral/gustatory responsive neurons in the amygdala, they will be used to enhance the breadth of discussions dealing with how somatosensory and visceral information reaches the amygdaloid complex.

It is also important to point out that some insular cortices and parietal regions in the rat have been given names that differ to those assigned to the primate and feline species however, the nomenclature used should not be problematic as many of these minor differences will be addressed in the discussion that follows. The reader should also be aware that minor discrepancies do arise from time to time as researchers use different names when discussing, or referring to similar brain regions of the rat that are involved in the somatosensory cascade to the amygdala. Thus, although the cortical and subcortical somatosensory cascades to the amygdala appear to be complicated and difficult to comprehend, they can still be easily understood as long as one clings to the notion that most begin in the parabrachial nucleus and in the thalamus and involve either a direct thalamo-amygdalar somatosensory pathway (i.e. subcortical) or a more indirect thalamo-cortico-amygdalar route (i.e. cortical).

Despite any differences in nomenclature it is important to point out the fact that the primary somatosensory region (S1) in the cat, monkey and rat does not send information to

the amygdala directly but rather indirectly through a series of stages or cascades that involve the participation of several secondary somatosensory areas of the parietal lobe and posterior insula cortex (McDonald, 1998; Friedman, Murray, O'Neill and Mishkin, 1986; Heath and Jones, 1971; Burton and Kopf, 1984; Russchen, 1982). In general, the primary somatosensory area (S1) in the rat sends efferents to the parietal association areas which in turn transmit somatosensory signals by innervating posterior portions of the insula and rhinal cortices (McDonald, 1998; Shi, 1995 in McDonald, 1998; Friedman, et al., 1986).

Similar somatosensory cascades appear in the cat and monkey and these are generally organized into dorsal and ventral elements that project to different regions along the route to the amygdala (Freidman, et al., 1986; Mesulam and Mufson, 1984; Burton and Kopf, 1984). For example, in both the cat and monkey S1 sends a dorsal projection that targets somatosensory association areas 5 and 7 which do not provide any direct input to the amygdala (Russchen, 1982a; Friedman, et al., 1986). In stark contrast, the ventral projection from S1 targets the secondary somatosensory area (S2) which then goes on to innervate the granular insular cortex, the caudal regions of the dysgranular insular area which is located in the posterior insular lobule, and the rhinal cortices (Mesulam and Mufson, 1984; Friedman, et al., 1986; McDonald, 1998). S2 also innervates areas Te2 and Te3 which are components of the auditory belt cortices, and these two regions in turn project to posterior portions of the insula cortex (McDonald, 1998; Ono, et al., 1995; Shi and Cassell, 1998a,b).

In the monkey and cat the granular and dysgranular insular cortices innervate dorsal regions of the lateral nucleus of the amygdala, the lateral subdivision of the central amygdaloid nucleus, and discrete portions of the basal nucleus (Mesulam and Mufson, 1984; Friedman, et al., 1986; Burton and Kopf; 1984; McDonald, 1998). More specifically, the granular insula (called the ventral parietal area in the rat) targets the dorsal portions of the lateral amygdaloid nucleus whilst, the dysgranular insula (called the parietal rhinal cortex in the rat) sends out diffuse, dense, and robust projections to the basal, central and lateral amygdaloid nuclei respectively (Mesulam and Mufson, 1984; Friedman, et al., 1986; Burton and Kopf; 1984; McDonald, 1998; Shi, 1995). It is important to point out that electrophysiological studies carried out on several mammalian species have repeatedly shown that the majority of somatosensory responsive neurons are localized in the lateral,

basolateral and central amygdaloid nuclear groups and their related subdivisions (Machne and Segundo, 1956; Creutzfeldt, Bell and Adey, 1963; Ono and Nishijo, 1992; Ono, et al., 1995; Uwano, et al., 1995). Typically, the lateral and basolateral nucleus of the amygdala and AStr which forms the ventral portions of the caudate-putamen contain the largest populations of somatosensory responsive neurons, however the mediolateral parts of the central nucleus of the amygdala contain substantial numbers of unimodal and multimodal somatosensory responsive neurons as well (One, et al., 1995; Uwano, et al., 1995).

The above electrophysiological data regarding somatosensory responsive amygdaloidal neurons is very compatible with the anatomical research since it indicates that the amygdala, in most species studied thus far, is the recipient of cortical and subcortical somatosensory information. Much of the cortical and subcortical somatosensory cascades to the amygdala involve various thalamic nuclei that either project directly to the amygdala (i.e. subcortical thalamo-amygdalar pathway) or begin by first projecting to S1 which initiates the cortical cascade by projecting to S2. This latter projection entails the indirect thalamo-cortico-amygdalar somatosensory pathway that was discussed earlier. In most mammalian species, including the rat, S1 receives somatosensory input from the ventral posterolateral and ventral posteromedial nuclei of the thalamus and then relays the information to S2 to start the somatosensory cortical cascade.

The direct subcortical somatosensory cascade to the amygdala involves the participation of various thalamic nuclei which include; the medial subdivision of the medial geniculate nucleus (mdMGN), the suprageniculate nucleus (SGN), and the posterior intralaminar nucleus (PIN). These three thalamic nuclei provide robust direct projections to the lateral and basolateral amygdaloidal nuclei and to the AStr with lighter projections targeting the central and basomedial amygdaloid nuclear groups (LeDoux, Farb, and Romanski, 1990; 1991; Turner and Herkenham, 1991). As was the case with auditory and visual thalamo-amygdalar pathways, the somatosensory thalamo-amygdalar projections arising out of mdMGN, SGN and PIN bifurcates and sends somatosensory efferents to Te2 and Te3. Association areas Te2 and Te3 in turn innervate the posterior insular regions which forward somatosensory information to the basolateral complex and the AStr (LeDoux, Farb and Romanski, 1991; Mascagni, McDonald and Coleman, 1993; Turner and Zimmer, 1984). This neuroanatomical organization or arrangement ensures that cortical and subcortical

somatosensory information has a high degree of overlap and convergence when it finally reaches the amygdala (see Figure 3). In addition, subcortically based somatosensory information also reaches the basomedial, central, and medial amygdaloid nuclei directly via the medial posterior nucleus of the thalamus and the parabrachial nucleus (Turner and Herkenham, 1991; LeDoux, Farb and Ruggiero, 1990; LeDoux, Farb and Romanski, 1991; Bernard, Alden and Besson, 1993).

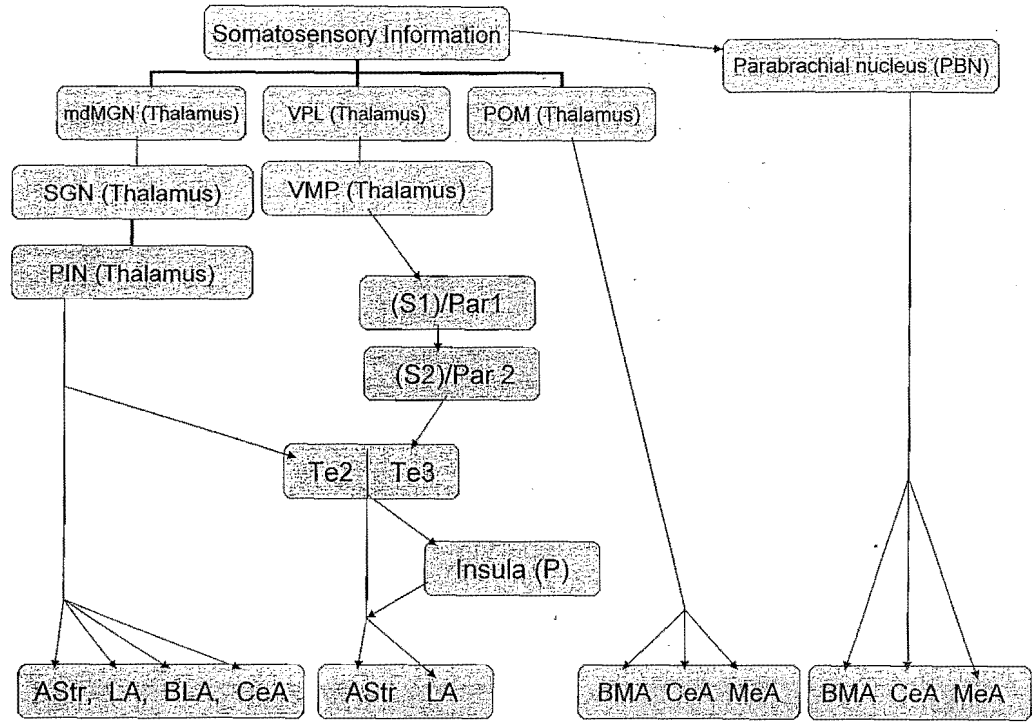

Figure 3 above provides a schematic depiction of how somatosensory information reaches the amygdala. Somatosensory information reaches the amygdala three or even four ways. The first is directly through the medial division of the medial geniculate nucleus of the thalamus (mdMGN), the suprageniculate nucleus of the thalamus (SGN), and the posterior intralaminar nucleus of the thalamus (PIN). mdMGN, SGN and PIN together send somatosensory input to the AStr, LA, BLA, and CeA. These thalamic nuclei also pass somatosensory information on to Te2. The second somatosensory cascade is indirect (i.e. via thalamo-cortico-amygdaloid pathways) whereby the ventral posterolateral nucleus of the thalamus (VPL) and the ventral posteromedial nucleus of the thalamus (VMP) send sensory information to the primary somatosensory area (S1/Par1). S1/Par1 sends information to the secondary somatosensory area S2/Par2. S2/Par2 in turn sends somatosensory information to Te3. Some of the information in Te3 is passed on directly to the amygdala (i.e. AStr and LA), whilst some is sent to the posterior insula cortex. The third somatosensory cascade originates from the medial posterior complex of the thalamus (POM) and sends information directly to the BMA, central nucleus of the amygdala (CeA) and medial nucleus of the amygdala (MeA). The final way somatosensory information reaches the amygdala (i.e. BMA, CeA and MeA) is directly via the pontine parabrachial nucleus (PBN).

3.42: Nomenclature in Neuroscience

Although the somatosensory cascade in the rat is generally quite similar to those observed in the cat and monkey, some of the terminology used to describe insular rhinal and parietal somatosensory association areas differs across species and may even be quite diverse when different neuroanatomists and physiologists study the functionality of these brain regions in the rat. For this reason, it is necessary to briefly mention some of the differences in terminology and nomenclature that occur from time to time before moving on to examine how visceral and gustatory information is transmitted to the amygdala. McDonald (1998) indicates that the primary somatosensory region (S1) of the rat projects to S2, the parietal ventral area (PVA), and the parietal rhinal cortex (PRHC) but not to the anterior aspects of the posterior agranular insular cortex (PAIC). The projection regions PVA and PRHC in the rat are generally considered to be analogous to the granular and dysgranular insular cortices found in the brains of cats and monkeys (McDonald, 1998). According to this scheme the caudal portions of PAIC, PRHC and PAIC located between -1.80 and -3.50 mm posterior to bregma were initially thought to form the anterior aspects of the PRh as described in the earlier second edition of the rat brain atlas produced by Paxinos and Watson (1986).

More recently however, several tracing studies along with more detailed anatomical assessments made by several researchers (Shi, 1995; in McDonald, 1998; Mascagni, et al., 1993; McDonald, 1998) including Paxinos and Watson (1998) have established that granular and dysgranular insular regions are also present in the rat brain and are found approximately -1.30 to -3.00 mm posterior to bregma and are just dorsal to the posterior agranular insular cortex, the ectorhinal and perirhinal cortices and anterior sections of the entorhinal cortex. In this new anatomical configuration of the rat brain, the entorhinal and perirhinal cortices are more caudally positioned and are rostrally contiguous with the posterior agranular insular cortex and certain aspects of the posterior parietal rhinal cortex of McDonald (1998) or the dysgranular insular cortex of Paxinos and Watson (1998).

The only notable difference between Paxinos and Watson's (1998) anatomical classification of the rat brain and that of McDonald (1998) is that McDonald includes the parietal ventral area and the parietal rhinal cortex as specialized somatosensory processing regions interposed between the more caudally situated PRh and the more rostrally located

granular, dysgranular and posterior agranular insular cortices. In contrast, Paxinos and Watson (1998) do not include the parietal ventral area and the parietal rhinal cortex in their classification scheme but rather have the granular, dysgranular and posterior agranular insular cortices forming the rostrodorsal borders of the PRh, the ectorhinal and entorhinal cortices, and the auditory association area Te3 (see McDonald, 1998 and Paxinos and Watson, 1998 for a comparison). Also, McDonald (1998) views the caudal half of the parietal rhinal cortex and the agranular insular cortex located between -1.80 mm to -3.50 mm posterior to bregma in the rat as part of the somatosensory insula. Regardless of the slight differences used in the classification of the rat brain, the granular and dysgranular insular structures in the cat and monkey are considered to be structurally and functionally equivalent to the ventral parietal area and parietal rhinal cortices in the rat (i.e. caudal granular and dysgranular insular regions of the rat according to Paxinos and Watson, 1998).

Anatomical evidence gathered from anterograde tracing studies in the rat indicates that input from S1 to the amygdala is light with more robust input coming from parietal association areas and the insula (McDonald, 1998). For example, PHA-L infusion into the primary somatosensory region (S1) produced virtually no anterograde labelled axon fibres in the amygdala (McDonald and Mascagni, 1998), whereas similar injections into the secondary association area (S2), and rostroventral parietal area produced a light to moderate labelling of axonal fibres that were confined to the magnocellular division of the basal nucleus and to the lateral capsular division of the central nucleus (McDonald, 1998). Injections of PHA-L into cells of the parietal ventral area produced moderate labelling of axon fibres in the dorsal and ventral region of the lateral nucleus of the amygdala and in the lateral capsular subdivision of the central nucleus when coronal sections at -2.00mm to 2.80mm from bregma were examined microscopically (McDonald, 1998). A slightly heavier labelling of fibres was also evident in the AStr and the ventral caudate putamen when coronal sections at these levels were studied. Generally speaking, the most robust projection to the amygdala was observed following PHA-L injections into the caudal regions of the ventral parietal area, PRh, or the posterior agranular insula cortex. Infusion of anterograde tracers into these three areas mentioned above produced labelled terminating fibres in the dorsolateral and ventrolateral subdivisions of the lateral nucleus, the basal

nucleus, the periamygdaloid cortex and the lateral capsular division of the central nucleus (McDonald, 1998).

3.43: From Somatosensory Cascades to Visceral and Gustatory Input to the Amygdala

The parietal rhinal cortex in the rat, which is referred to as the dysgranular insular area in the monkey, projects to the ventral portions of the PRh (McDonald, 1998), however a great number of projections are also directed towards the amygdala. For example, studies incorporating PHA-L tracing methods have shown that the posterior insula and rhinal cortices provide a vigorous innervation of several amygdaloidal nuclei, with the ventral parietal area in the rat targeting the dorsal and ventral portions of the lateral amygdaloid nucleus, while the parietal rhinal cortex sends out diffuse, dense, and robust projections to the central, basal and lateral amygdaloid nuclei (McDonald, 1998; Shi, 1995 in McDonald, 1998; Shi and Cassell, 1998a). Furthermore, results obtained from PHA-L injections made into the parietal rhinal cortex and the caudal sector of the posterior agranular insular cortex in the rat provide even more compelling anatomical evidence that somatosensory information reaches the central amygdaloid nucleus and much of the basolateral complex as most of the labelled fibres were found in the ventrolateral aspects of the basal and accessory basal amygdaloid nuclei (McDonald, 1998; also see Shi and Cassell, 1998a). It is important to note that the majority of the labelled axons found in the ventral aspects of the basal amygdaloid nucleus were located in the parvicellular subdivision of the basal nucleus. Similar aggregations of labelled axon terminating fibre systems also appeared in the endopiriform nucleus located just ventral to the parvicellular subdivision of the basal nucleus, while fewer numbers were observed in the central nucleus of the amygdala (McDonald, 1998).

Larger numbers of anterograde labelled cell fibres appeared in the central nucleus of the amygdala only when PHA-L infusions were made into rostral portions of the dysgranular insular cortex (i.e.-0.2 mm from bregma) in an area just medial to the rhinal fissure and slightly dorsal to the posterior agranular insular cortex (McDonald, 1998). Most of this labelling appeared to be confined to the lateral and medial subdivisions of the central amygdaloid nucleus however some moderate labelling was localized to the lateral extremities of the dorsal subdivision of the lateral nucleus and lateral portions of the

posterior basolateral amygdaloid nucleus (McDonald, 1998). It is important to point out that anatomical and electrophysiological research carried out on the rat, as well as the monkey, cat, and rabbit indicate that a great deal of cells located in the more rostral portions of the granular, dysgranular and agranular insular cortices are involved in transmitting gustatory and visceral sensory information to the lateral, basal and central amygdalar nuclear groups (Mufson et al., 1981; Turner, et al., 1980; Yaxley, et al., 1990; Plata-Slaman, et al., 1992; 1993; Scott, et al., 1993; 1994; Aggleton, et al., 1980; Ono and Nishijo, 1992; Yasui, et al, 1983; 1987; 1991; Pascoe and Kapp, 1987; Oppenheimer and Cechetto, 1990; Allen, et al., 1991; McDonald, 1998; Shi, 1995; Korn, 1969; Ono, et al., 1995; Uwano, et al., 1995). Hence, more anterior located insular regions in most mammalian species (i.e. approximately -0.2 to + 0.3 mm from bregma in the rat) may represent a transition zone where somatosensory cortical cascades to the amygdala come to an end and where visceral sensory and gustatory cascades to the amygdala begin.

Several lines of scientific evidence tend to strengthen the transition zone argument or at the very least provide strong support for the general view that the anterior insular region (gustatory/visceral) can be structurally and functionally separated from the posterior insular regions involved in the somatosensory cascade to the amygdala. First, research has demonstrated that anterior aspects of the insula contains both gustatory and viscerally responsive neurons that are significantly more responsive to food and saccharin solutions and to conditioned stimuli that predict the onset of these primary reinforcers and are less responsive to somatosensory cues (Yaxley, et al., 1990; Plata-Salaman, et al., 1992; 1993; Scott, et al., 1994; Korn, 1969; Azuma, et al., 1984; Ono, et al., 1995; Uwano, et al., 1995). Second, stimulation of the anterior dysgranular and posterior agranular regions produces profound changes in heart rate, blood pressure and gastric motility (Oppenheimer and Cechetto, 1990; Yasui, et al., 1991) and was shown to significantly alter the activity level of approximately seventy percent of neurons located in the central nucleus of the amygdala (Pascoe and Kapp, 1987), thus indicating that anterior regions of the insula are involved in both visceral sensory and visceral motor functions. In this connection, it is also noteworthy that amygdaloid neurons located in the lateral, basal, and magnocellular subdivision of the basal nucleus which receive input from the anterior insular regions, were shown to increase their activity levels in response to the ingestion of food and liquid refreshments (Scott, et

al., 1993; Ono and Nishijo, 1992). Third, the general arrangement and location of both gustatory and viscerally responsive neurons in the amygdala tends to correspond well with the terminal projection fields of cells in the gustatory and visceral insular regions that have been mapped by anterograde and retrograde tracing methods (Azuma, et al., 1984; Shi, 1995; Yasui, et al., 1991; Ottersen, 1982; Allen, et al., 1991). For example, most gustatory and viscerally responsive neurons in the lateral, basal and central amygdaloid nuclei along with those located in the cortical and medial amygdaloid nuclei receive cortical projections from the anterior granular, dysgranular and agranular areas of the insula (Mufson, et al., 1981; Azuma, et al., Mascagni and McDonald in McDonald, 1998; Ono, et al., 1995). These three anteriorly situated insular cortices are the recipients of gustatory and visceral information that arrives at the dorsomedial and ventrolateral aspects of the parvicellular subdivision of the ventroposterior medial thalamic nucleus via the rostral and caudal portions of the solitary nucleus respectively (Mesulam and Mufson, 1984; Beckstead, et al., 1980).

In marked contrast, most somatosensory information shunted to the more posterior portions of the insula (i.e. caudal posterior agranular insular area) and the parietal rhinal cortex arrives either via the posterior and ventral posterior thalamic nuclei or via the centrolateral nucleus of the thalamus but not by the parvicellular subdivision of the ventroposterior medial thalamic nucleus (McDonald, 1998). This somatosensory information in the posterior portions of the insula and the parietal rhinal cortices is then channelled to various amygdaloid nuclei to complete the somatosensory cascade. This indicates that the visceral and gustatory input to the anterior insula (granular, dysgranular and agranular areas) is transmitted to the amygdala by a completely different group of thalamic relay nuclei than those that distribute somatosensory information to the parietal rhinal cortex, caudal agranular insular area and various amygdaloid nuclei (see Figure 4).

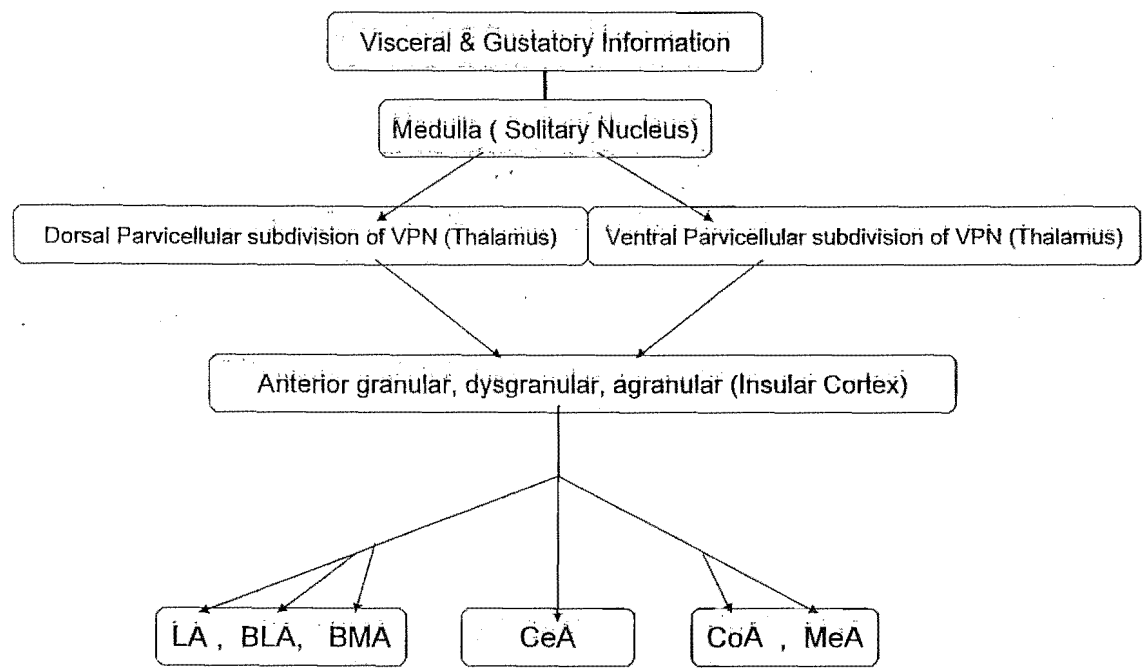

Figure 4. The above figure portrays a schematic representation of how visceral and gustatory information reaches the amygdaloid complex. Basically, the visceral and gustatory information cascade to the amygdala begins with the solitary nucleus of the medulla transmitting visceral and gustatory information to the dorsal parvicellular subdivision of the ventral posterior nucleus of the thalamus (dVPN) and the ventral parvicellular subdivision of the ventral posterior nucleus of the thalamus (vVPN). Visceral and gustatory information from the dVPN and vVPN is then sent to the anterior granular, dysgranular and agranular insular cortex. From these regions of the insula cortex, much of the visceral and gustatory information is sent to several amygdaloid nuclear groups. These include the LA, BLA, BMA and CeA. The insula cortex also sends visceral and gustatory information to the anterior cortical nucleus of the amygdala (CoA) and the medial nucleus of the amygdala (MeA).

3.5: Convergence of Sensory Input in the Amygdala and Some Implications

Although several types of modality specific sensory information that reach the amygdala can generally be dissociated from one another, it is essential to understand that the convergence of somatosensory, visceral/gustatory, visual, auditory, olfactory and nociceptive information occurs in the lateral, basolateral and central amygdaloid nuclei (Machne and Segundo, 1956; Creutzfeldt, et al., 1963; Uwano, et al., 1995; Ono, et al., 1995; Romanski and LeDoux, 1993; Shi and Davis, 1996; 1997; 1999; Switzer, De Olmos, and Heimer, 1985; see McDonald, 1998; Shi and Davis, 2001) and has been shown to play a vital role in both fear learning and in an animal's capacity to evaluate and respond to biologically significant appetitive and aversive motivational stimuli (Uwano, et al., 1995; Ono, et al., 1995; Davis, 1992a,b,c; LeDoux, et al., 1988; LeDoux, 1993; Koch, 1999; Fendt and Fanselow, 1999; Shi and Davis, 1997; 1999; 2001). As a matter of fact, a few early pioneering research efforts made decades ago demonstrated that extensive somatosensory, olfactory and auditory convergence takes place in the basolateral and central amygdaloid nuclei (Machne and Segundo, 1956; Creutzfeldt, et al., 1963). More recently, electrophysiological assessments by Romanski and colleagues (1993) demonstrated that neurons located in the dorsolateral subdivision of the lateral amygdaloid nucleus respond to footshock and acoustic stimuli. Additionally, several paradigms that measure amygdala-mediated Pavlovian fear conditioning and fear-motivated learning indicate that visual and auditory cues that are paired with either footshock (i.e. somatosensory/nociceptive) or noxious food and liquid solutions (visceral/gustatory/olfactory) may become linked by convergence that occurs in the basolateral and central amygdaloid nuclei (Ono, et al., 1995; Uwano, et al., 1995; Romanski, Clugnet, Bordi and LeDoux, 1993; LeDoux, Cicchetti, Xagoraris and Romanski, 1990b). Generally speaking, the defensive freezing, FPS and conditioned taste aversion paradigms have been quite successful in not only ascertaining how sensory information from several sensory modalities (both nociceptive and non-nociceptive) reaches various amygdaloid nuclei, but also how the convergence of these cortical and subcortical cascades in the basolateral complex contributes to fear learning and expression when distinct sensory cues are manipulated during the training and testing phases of experiments (Shi and Davis, 1996; 1997; 1999; 2001; Romanski and LeDoux, 1993; Romanski, Clugnet, Bordi and

LeDoux, 1993; LeDoux, et al., 1990b;Yamamoto, Fujimoto, Shimura and Sakai, 1995; Yamamoto, Azuma, and Kawamura, 1984; Lasiter and Glanzman, 1985; Ono, et al., 1995; Uwano, et al., Ferry, Sandner, and Di Scala, 1995; LeDoux and Muller, 1997).

To summarize, the results obtained from anterograde tracing experiments conducted on the rat (Shi, 1995; in McDonald, 1998; McDonald and Mascagni, in McDonald, 1998) are generally consistent with those obtained from similar experimentation carried out on the monkey (Mufson, et al., 1981; Friedman, 1986) in that they seem to indicate that somatosensory information from S1 and S2 is channelled through the parietal rhinal cortices and the granular, dysgranular and posterior agranular insular regions before reaching lateral, basolateral or central amygdaloid nuclei. For example, Mufson, et al., (1981) demonstrated that injections of titrated amino acids into the granular and dysgranular regions of the posterior insula produced a robust anterograde labelling of axon fibres in the dorsal lateral amygdaloid nucleus with smaller amounts found in the amygdala's central nucleus. Similarly, Friedman, et al., (1986) showed that horse radish peroxidase infused into the granular insular cortex caused anterograde labelling in the lateral nucleus of the amygdala. In a similar vein, both Shi (1995) and McDonald (1998) used different anterograde tracing techniques to establish that neurons in the rat's parietal ventral area, posterior rhinal cortex, and caudal portions of the insular cortex (i.e. posterior agranular insula) project to the lateral, basolateral and central amygdaloid nuclei.

Based on the work of Shi (1995; in McDonald, 1998), McDonald, (1998) and various others (Machne and Segundo, 1956; Creutzfeldt, et al., 1963; Turner and Zimmer, 1984; Yasui, et al., 1991; Azuma, et al., 1984; Scott, et al., 1993; Ono and Nishijo, 1992; Schneider, et al., 1993; Pascoe and Kapp, 1987; Ono, et al., 1995; Uwano, et al., 1995) it has been determined that the somatosensory cascade to the amygdala can be anatomically and electrophysiologically dissociated from the visceral sensory and gustatory cascades since it involves different thalamic relay nuclei and efferent pathways (Beckstead, et al., 1980; Pritchard, et al., 1986) and distinct cortical substrates. As pointed out above, somatosensory cortical cascades to the amygdala arise primarily from the parietal ventral area, the parietal rhinal cortex and more caudal insular cortices. On the other hand, gustatory and visceral projections to the amygdala arise from more anterior insular regions (i.e. anterior granular, dysgranular and agranular insular cortices) and this seems to be the

case in the rat (Shi, 1995; Yasui, et al., 1991; Mascagni and McDonald in McDonald, 1998; Ottersen, 1982; Allen, et al., 1991), cat (Yasui, et al., 1987), monkey (Mufson and Mesulam, 1984; Mufson, et al., 1981; Mesulam and Mufson, 1992) and human (Mufson and Mesulam, 1984; Mesulam and Mufson, 1992). These gustatory and visceral cortical regions, like their somatosensory counterparts generally have strong projections to the dorsal subdivision of the lateral nucleus, the central nucleus of the amygdala and selective portions of the basal and accessory basal amygdaloid nuclei (Aggleton, et al., 1980; Mufson, et al., 1981; Turner, et al., 1980; Yasui, et al., 1991; McDonald, 1998; Yasui,, et al., 1987) and a wealth of research indicates that learned associations are established by sensory convergence taking place in the basolateral complex (Ono, et al., 1995; Uwano, et al., 1995; Romanski, Clugnet, Bordi and LeDoux, 1993; LeDoux, et al., 1990; for reviews see LeDoux, 1993; Maren, 1999; Fendt and Fanselow, 1999).

The research evidence demonstrating that somatosensory information finds its way mostly to the lateral, central, and BLA is particularly important since somatosensation in its extreme form is noxious and can result in pain or discomfort. Thus, certain types of somatosensory stimuli when painful, can act as bodily cues that inform the amygdala and the rest of the fear circuitry that harm is being caused and defensive action needs to be taken. The same rule applies to stimuli (i.e. toxic food liquid solutions and drugs, as well as stressors such as footshock and isolation) that produce unpleasant visceral and gustatory reactions and ahedonic internal bodily states such as nausea, sickness, increased heart rate and blood pressure, gastric motility and respiratory distress. Processing of these aversive visceral and gustatory reactions by the amygdala teaches the organism which experiences them to avoid environments, food sources and stimuli that predict illness or stress. Thus, when aversive somatosensory cues (i.e. nociceptive input; e.g. footshock) are repeatedly paired with salient neutral visual cues (i.e. light), contextual cues (i.e. test apparatus) and various internal ahedonic bodily states (i.e. visceral and gustatory reactions to unpleasant stimuli), they can all combine to support and facilitate Pavlovian fear conditioning which produces an indelible fear-memory trace in the neural substrates of the amygdala-based fear system.

3.51: Nociceptive cortical and subcortical pathways to the Amygdala

Numerous cortical and subcortical brain systems are involved in the processing and distribution of nociceptive information to the amygdala and there generally seems to be a great deal of overlap between indirect cortico-amygdalar pain pathways and the more direct subcortico-amygdalar pain pathways as far as the transmission of nociceptive information to the amygdala is concerned (Craig, Krout and Zhang, 1995; Dostrovsky and Craig, 1996; LeDoux, Ruggierro and Reis, 1985; Shi, 1995; Shi and Davis, 1997; 1999; Romanski and LeDoux, 1990). The pathways that carry somatosensory information to the amygdala are very important because many of the areas involved in this somatosensory cascade to the amygdala also process nociceptive information that is a prerequisite for Pavlovian and instrumental fear conditioning paradigms which employ footshock as an aversive UCS (Shi and Davis, 1997; 1999; Romanski and LeDoux, 1993; LeDoux, 1993).

Thus, brain regions such as the ventral parietal area, the secondary somatosensory area, and the granular and dysgranular insular regions including some parts of the PRh that target the amygdala are also involved in pain information processing and transmission. For example, several anatomical, electrophysiological and lesioning experiments carried out on primates and rats have discovered that neurons in the dorsal insular region next to the secondary somatosensory area (S2) play a prominent role in nociceptive information processing (Craig, Krout and Zhang, 1995; Dostrovsky and Craig, 1996; Shi and Davis, 1997; 1999). Similarly, experiments designed to elucidate the cortical areas involved in pain processing in humans have revealed that application of painful stimuli to the skin causes neurons in S2 and some nearby adjacent insular regions to become more metabolically active (Talbot, Marrett, Evans, Meyer, Bushnell and Duncan, 1991), thus providing strong evidence that S2 and the dorsal insular region are involved in processing nociceptive information.

These experimental results obtained from humans and primates seem to apply to other mammalian species found lower on the phylogenic scale as well, since the administration of the type I herpes simplex virus to the tooth pulp of rats (an anatomical area strongly innervated by nociceptive neurons) was shown to produce a significant transneuronal anterograde transport of the herpes virus to both the secondary somatosensory area and parietal rhinal cortex (Barnett, Evan, Sun, Perlman and Cassell, 1995). However, some

caution should be taken when applying the results of the Barnett, et al., (1995) study to all forms of central nervous system pain processing since the study in question focused primarily on nociceptive information being routed via the trigeminal pathway to the cortex which likely involves the participation of the caudal spinal trigeminal nucleus which processes pain and temperature information from the mandibular, maxillary and ophthalmic regions of the head (Nolte, 1993) and not on the nociceptive information that arrives from the appendages and digits via the spinothalamic tract. Nevertheless, research by Willis, Westland and Carlton (1995) has demonstrated that the posterior thalamus (which includes the posterior thalamic nucleus, the medial subdivision of the medial geniculate nucleus and the posterior intralaminar nucleus) is a major recipient of nociceptive information stemming from the spinal cord and trigeminal nuclear groups, and since the thalamus is intimately connected to both the amygdala and many of the cortical areas that are involved in the somatosensory cascade (Ottersen, and Ben-Ari, 1979; Russchen, 1982b; LeDoux, et al., 1985; 1987; 1990; Turner and Herkenham, 1991; Shi, 1995; Bordi and LeDoux, 1994) it is highly probable that nociceptive information reaches the amygdala in a variety of ways.

In general, nociceptive information is routed to the amygdala at least three different ways and along three distinct pathways which may at times overlap to target similar amygdaloidal and cortical regions. The first way is the indirect route via thalamo-cortico-amygdalar and cortico-amygdalar pathways that transmit nociceptive information through the thalamus and cortical regions such as S2, the parietal ventral area, the parietal rhinal cortex and the PRh to the basolateral amygdaloid complex (Craig, et al., 1995; Dostrovsky and Craig, 1996; Barnett, et al., 1995; Shi and Davis, 1997; 1997; Romanski, Clugnet, Bordi, and LeDoux, 1990). The second way is more direct via the thalamo-amygdalar pathways that involve the medial subdivision of the medial geniculate nucleus, the posterior intralaminar nucleus and the posterior nucleus (Romanski, et al., 1990; Shi and Davis, 1997; 1999). The third way involves the ponto-parabrachial-amygdalar nociceptive pathway which transmits information about noxious and painful stimuli directly to the lateral capsular subdivision of the central amygdaloid nucleus, the basolateral complex, and the AStr via a relay located in the midbrain parabrachial nucleus (Bernard and Besson, 1988; Bernard, Huang, and Besson, 1992; Bernard, Peschanski, and Besson, 1989; Slugg and Light, 1994).

Indeed, numerous electrophysiological and anatomical studies have shown that parabrachial and thalamic efferents are involved in transmitting nociceptive information to the amygdala (LeDoux, et al., 1987; Bernard and Besson, 1988; Bernard, et al., 1989; 1992; Romanski, et al., 1990; Slugg and Light, 1994; Shi and Davis, 1997; 1999) and it is important to point out that these two subcortical nuclei project to many of the same amygdaloid nuclei that are the recipients of both somatosensory and nociceptive input coming from such brain regions as the secondary somatosensory area, the posterior insular cortex, the parietal ventral area, the parietal rhinal cortex, the granular and dysgranular insular cortices, and some portions of the PRh (Shi and Davis, 1997; 1999; Ono, et al., 1995; Uwano, et al., 1995; Shi and Cassell, 1998a,b; Shi and Cassell, 1999). For example, the dorsolateral subdivision of the lateral amygdaloid nucleus receives somatosensory and nociceptive information from the medial subdivision of the medial geniculate nucleus of the thalamus and the posterior intralaminar nucleus of the thalamus in conjunction with similar input arriving from the parietal ventral area and the parietal rhinal cortex (LeDoux, et al., 1990; Romanski, et al., 1993; Bordi and LeDoux, 1994).

Similarly, the lateral capsular subdivision of the central amygdaloid nucleus and the AStr also receive nociceptive input from the parabrachial nucleus, the medial subdivision of the medial geniculate nucleus of the thalamus, and the posterior intralaminar nucleus of the thalamus, and these amygdaloid nuclear groups are also at the receiving end of cortical projections emanating from the parietal ventral area and the parietal rhinal cortical fields, two regions that are involved in processing somatosensory and nociceptive information (Bernard and Besson, 1988; 1990; Bernard, et al., 1993; Shi and Davis, 1997; Shi and Davis, 1999; Shi and Cassell, 1998a; McDonald, 1998). Moreover, the posterior nucleus of the thalamus has robust projections to the central amygdaloid nucleus and the anterior subdivision of the cortical nucleus and receives nociceptive input from the trigeminal nuclear group and the spinothalamic tract (LeDoux, Ruggiero, Forest, Stornetta and Reis, 1987; Bordi and LeDoux, 1994). Recent, anatomical and lesion experiments conducted by Shi and Davis (1997; 1999) are consistent with numerous other studies that have examined how nociceptive information finds its way to the amygdala (Romanski, et al., 1990; Shi and Davis, 1997; Ono, et al., 1995; Turner and Herkenham, 1991) and the results of Shi and Davis (1997; 1999) provide additional support to the notion that the amygdala receives

nociceptive input through at least two and perhaps even three parallel pathways. For example, Shi and Davis (1997; 1999) suggested that footshock information travelling through the spinal cord is relayed directly to the basolateral amygdaloid complex via a thalamo-amygdala pathway that first synapses in the PIN. Based on their work in the FPS paradigm Shi and Davis (1997; 1999) then went on to conclude that a second indirect UCS pain pathway to the basolateral complex involves relays in the ventral posterior thalamic nucleus, the posterior thalamic nucleus, and the posterior intralaminar nucleus transmitting nociceptive information to S1, S2, and the caudal insular region (see Figure 5). This second indirect pain pathway mapped by Shi and Davis (1997; 1999) is for the most part, identical to the thalamo-cortico-amygdaloid nociceptive pathway that has already been discussed in this section.

In closing, it is necessary to emphasize the important role of cortical and subcortical nociceptive information processing and transference to the amygdala along with the wealth of sensory convergence that occurs in the lateral and basolateral amygdaloid nuclei. Sensory convergence when combined with cortically and subcortically processed nociceptive information cascades to the amygdala may serve to not only provide the amygdala with the vital informational clues about the discriminative features or qualities of pain such as its intensity, duration, and location but it may also help with the formation and maintenance of strong and long-lasting CS-UCS associations observed following Pavlovian fear conditioning. Thus, the convergence of sensory information in the amygdala that results after repeated pairings of salient auditory/visual sensory cues with aversive footshock conceivably produces long term changes in the amygdala-based fear system that causes fear-memories to be so enduring and fear expression to be so potent.

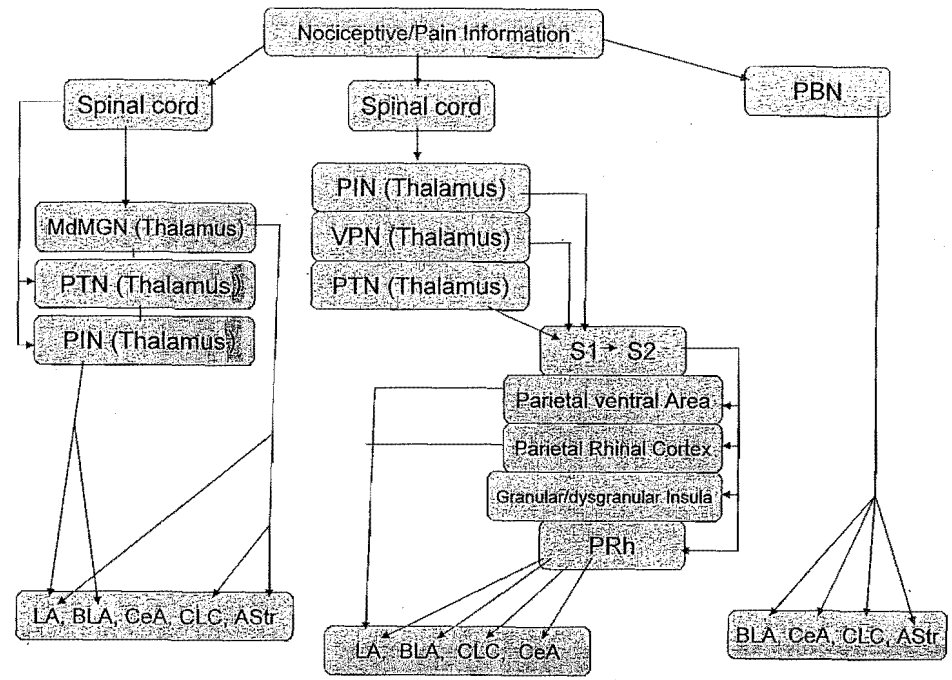

Figure 5 (above) displays the three general ways nociceptive information reaches the amygdala complex. The first (depicted on the left) is the direct spino-thalamo-amygdaloid pain pathway. This pathway involves pain signals in the spinal cord being transmitted to several thalamic nuclei. These include the medial division of the medial geniculate nucleus (mdMGN), the posterior thalamic nucleus (PTN) and the posterior intralaminar nucleus (PIN). The mdMGN passes on pain information to the AStr, LA and the lateral capsular subdivision of the central amygdaloid nucleus (CLC). Similarly PTN and PIN transmit nociceptive information directly to the LA and BLA. The second direct pain pathway to the amygdala involves the parabrachial nucleus (PBN) that sends information to the BLA, CeA, CLC and AStr (see right side of figure). The third nociceptive pathway is the spino-thalamo-cortico-amygdaloid system depicted in the middle of Figure 5. PIN, PTN and VPN (ventral posterior nucleus of thalamus) receive input from the spinal cord and then pass this on to the primary somatosensory area S1. S1 sends pain information to the secondary somatosensory region (S2). Through a series of cascades S2 sends nociceptive information on to the parietal ventral area, the parietal rhinal cortex, the posterior granular and dysgranular insula, and the perirhinal cortex (PRh). Finally, several amygdaloid nuclei (i.e. LA, BLA, CLC, and CeA) receive nociceptive information from the PRh and parietal regions.

3.6: Perirhinal Cortex Projections to the Amygdala

The PRh is a structurally and functionally distinct region that receives a wealth of highly processed cortical input from virtually every sensory modality and it is intimately connected to the amygdala (McDonald and Mascagni, 1996; Shi and Cassell, 1999; Shi and Cassell, 1998a). Studies employing PHA-L anterograde tracing techniques have shown that the PRh projects primarily to the lateral nucleus and the AStr with a moderate to light labelling of neurons in the accessory basal and basal nuclei respectively (Romanski and LeDoux, 1993). Also, anatomical evidence gathered from several laboratories over the years indicates that the PRh is made up of a dorsal region which receives visual and auditory input from various temporal association areas (Mascagni, et al., 1993; Romanski and LeDoux, 1993; Shi and Cassell, 1997a; McDonald and Mascagni, 1996) and a ventral region which receives somatosensory information from S2 and the posterior insular cortices (Shi and Cassell, 1997a,b in McDonald, 1998; Shi and Cassell, 1999), nociceptive information from the dorsal insula (Craig, Krout and Zhang, 1995; Dostrovsky and Craig, 1996) and a wealth of highly processed polymodal limbic information from the prefrontal cortex (Decon, Eichenbaum, Rosenberg and Eckman, 1983; Shi and Cassell, 1997b in McDonald, 1998; Shi and Cassell, 1999).

This distinct functional and anatomical organization of the PRh makes it highly probable that the amygdala would be the recipient of selective and highly processed information coming from different regions in the PRh. Shi and Cassell (1999; 1997b in McDonald, 1998) used biocytin injections into the dorsal and ventral portions of the PRh to investigate whether or not these two regions targeted different amygdaloid nuclei. In general the results of Shi and Cassell (1997b in McDonald, 1998; also see Shi and Cassell, 1999) were congruent with previous tracing experiments which mapped the organization and localization of perirhinal projections to the amygdala (Mascagni, et al., 1993; Romanski and LeDoux, 1993; Turner and Zimmer, 1984; Shi and Cassell, 1997a) however, the main addition this time was the confirmation that the dorsal and ventral banks of the PRh do indeed innervate different amygdaloidal regions and that this projection is topographic in nature. For example, biocytin infused into the dorsal PRh produced numerous labelled fibres in the lateral nucleus of the amygdala and in the dorsal portion of the magnocellular division of the basal nucleus (Shi and Cassell, 1997b; in McDonald, 1998; Shi and Cassell,

1999). Moderate labelling was also observed in the accessory basal nucleus, the lateral capsular division of the central nucleus and in the periamygdaloid cortex (Shi and Cassell, 1997b in McDonald, 1998; Shi and Cassell, 1999).

In marked contrast, similar infusions of biocytin into the ventral bank of the PRh was observed to produce a robust labelling of fibres and cell bodies in the anterodorsal and anterolateral regions of the basal nucleus with a very light sprinkling of labelled fibres and cells confined to the anterior tip of the lateral amygdaloid nucleus (Shi and Cassell, 1997b in McDonald, 1998; Shi and Cassell, 1999). Additionally, biocytin infusions made into the border area between the dorsal and ventral perirhinal regions at the level of the fundus of the rhinal sulcus generated labelled fibres in the lateral capsular subdivision of the central nucleus and in the lateral nucleus with some overlapping occurring in the basal nuclei as well (Shi and Cassell, 1997b in McDonald, 1998; Shi and Cassell, 1999).

In general, the topographical arrangement or nature of PRh projections to the amygdala can be summarized as follows. First, projections to the accessory basal amygdaloid nucleus and the periamygdaloid cortex originate from cells located in the very dorsal aspects of the dorsal PRh. Second, projections to the basal amygdaloid nucleus arise primarily from cell groups located in the ventral banks of the PRh in an area ventral to the rhinal sulcus. Finally, projections to the lateral amygdaloid nucleus and lateral capsular subdivision of the central nucleus can be traced back to projection neurons found in either the medial portion of the dorsal PRh or in the junction/transition zone between the dorsal and ventral perirhinal cortices near the rhinal sulcus which anatomically divides the two regions (Shi and Cassell, 1997b in McDonald, 1998; Shi and Cassell, 1999).

Taking this information into account, it is likely that the transmission of polymodal (i.e. auditory, visual, somatosensory and nociceptive) information to the amygdala via the perirhinal cortices ensures that the amygdala has all the sensory precursors and elements necessary for fear learning and memory storage. This notion is supported by the research efforts of McDonald and Mascagni (1996) who examined the projection fields of visual association area neurons that innervate the PRh. Based on their work, McDonald and Mascagni (1996) found that infusion of the anterograde tracer PHA-L into the medial and lateral portions of the secondary occipital cortex produced a substantial labelling of fibres that were confined to a narrow band of cortex located just dorsal to the rhinal sulcus and

near the area that demarcates the border between the more anterior parietal rhinal cortex and the more caudal PRh. This narrow band of specialized visual association cortex is typically found between -2.80mm and -4.00mm posterior to bregma in the rat and appears to be roughly analogous to the anterior sylvian area which is a visual processing association area located near the caudal end of the insula cortex in the cat (Clasca, Liamas and Reinoso-Suarez, 1997; McDonald and Mascagni, 1996; McDonald, 1998).

Furthermore, both electrolytic and fibre sparing neurotoxic lesions confined to the caudal PRh, adjacent temporal association areas, and/or the narrow band of specialized association cortex located at the junction of the parietal rhinal cortex and the PRh (i.e. an area covering -2.30 mm to -6.30 mm posterior to bregma) blocked FPS and defensive freezing behaviour when auditory, visual, and contextual cues were used to elicit fear responding (Rosen, et al., 1992 Corodimas and LeDoux, 1995; Campeau and Davis, 1995), thus clearly demonstrating that the flow of sensory information from the PRh and nearby association areas to the amygdala is essential for fear acquisition, expression and emotionally motivated responding. These lesion results, when combined with the data obtained from anatomical and electrophysiological studies of PRh sensory projections to the amygdala, indicate that the PRh not only receives and processes auditory and visual stimuli, but also acts as a neural substrate that supplies the lateral and BLA with highly processed information from several significant sensory modalities (i.e. auditory, visual, somatosensory and nociceptive). Since sensory convergence has been shown to occur in the basolateral complex, the information flow from the PRh, entorhinal cortex, hippocampus, insula and other temporal lobe regions that collectively make up the medial temporal lobe memory system ensure that the amygdala has all the necessary sensory precursors and elements to support fear learning and memory storage and to make fear retrieval and expression possible (Machne and Segundo, 1956; Creutzfeldt, et al., 1963; Romanski and LeDoux, 1993; Shi and Davis, 1996; 1997; 1999; Shi and Cassell, 1998a,b; Shi and Cassell, 1999; McDonald, 1998; Ono, et al., 1995; Uwano, et al., 1995).

3.7: Medial Prefrontal Cortex Projections to the Amygdala

Research using anterograde and retrograde tracing techniques has revealed that many amygdaloid nuclear groups receive input from axons emanating from areas of the medial

prefrontal cortex (Aggleton, et al., 1980; Carmichael and Price, 1995; McDonald, Mascagni, and Guo, 1996). For example, McDonald and colleagues (1996) used the anterograde tracer PHA-L to demonstrate that the infralimbic (IL) and prelimbic (PL) regions of the medial prefrontal cortex (mPFC) provide the amygdaloid complex with robust innervation. More specifically, McDonald and his co-workers (1996) showed that PHA-L injections made into the IL region caused labelled terminating axons to appear primarily in the lateral capsular subdivision of the central nucleus, the ventral subdivision of the lateral amygdaloid nucleus, the accessory basal nucleus, the dorsal aspects of the basolateral nucleus, and the medial nucleus of the amygdala. In start contrast, PHA-L infusions made into the PL region of the mPFC caused labelled axons to be generated mostly in the magnocellular division of the basolateral nucleus of the amygdala (McDonald, et al., 1996). However, these researchers did report that some terminating fibres could be seen scattered throughout the lateral amygdaloid nucleus and the AStr after PHA-L infusion into the PL region (McDonald, et al., 1996).

Additional research by Brinley-Reed, Mascagni and McDonald (1995) indicates that efferents emerging from the PL region of the mPFC usually target the dendritic spines of basolateral nucleus pyramidal neurons and portions of the lateral capsular subdivision of the central amygdaloid nucleus (CLC). It is also important to report that connections between the mPFC and the amygdala are topographically organized and reciprocal in nature (Carmichael and Price, 1995; McDonald, 1998). Thus, the amygdala not only receives robust input from the mPFC but also sends extensive projections back to the prefrontal cortical region (Krettek and Price, 1977a; Kita and Kitai, 1990; McDonald, 1991). Furthermore, research has shown that mPFC projections to the amygdala are largely glutamatergic in nature and that lateral and basolateral amygdaloid neurons contain large populations of glutamate receptors (i.e. NMDA and AMPA) that help to regulate the flow of excitatory neurosynaptic transmission within the amygdala and its many pathways (Amaral and Insausti, 1992; Farb, Aoki, and LeDoux, 1995; Rainnie, et al., 1991a; Davis, Rainnie and Cassell, 1994; Li, Phillips, and LeDoux, 1995; Gean and Chang, 1992; Gean, et al., 1993a; Maren, 1996; Wang, et al., 2001; 2002; Sah and De Armentia, 2003). Since the axons of mPFC neurons form synaptic contacts with the dendritic spines of pyramidal neurons in the BLA and CLC, it is possible that the mPFC may regulate the responsiveness

of amygdaloid neurons during fear conditioning and during extinction learning when amygdaloid cells would have to make adaptations to deal with the new information arising from changes in reinforcement patterns (i.e. repeated CS presentations with no footshock). Thus, the functional and anatomical relationship between the mPFC and amygdala may be important to laboratory animals as they make the transition from conditioned fear learning and expression to fear-extinction and behavioural inhibition. This notion is supported by electrophysiological work which shows that mPFC unit responses undergo changes with repeated presentations of conditioned fear cues and that BLA neurons control some of these fear-induced changes in mPFC neuronal activity (Baeg, Kim, Jang, Kim, Mook-Jung, and Jung, 2001; Garcia, Vouimba, Baudry, and Thompson, 1999; Pérez-Jaranay and Vives, 1991). Further evidence highlighting the importance of mPFC-amygdala connectivity comes from the work of Quirk, Likhtik, Pelletier and Pare (2003) who demonstrate that stimulation of the mPFC reduces the responsiveness of neurons in the central amygdaloid nucleus. On the basis of the above discussion, it is clear that mPFC-amygdala interconnectivity has the potential to influence the directionality of stimulus-stimulus associations during fear conditioning, CS presentations, and fear-extinction training sessions. Thus, mPFC innervation of the amygdala adds to and bolsters the vast array of cortically processes information that reaches the amygdala from other cortical brain regions.

3.8: Neural Pathways Exiting the Amygdala: Amygdaloid Efferents

While it is abundantly clear that afferent connections to the amygdala provide it with various forms of sensory input needed for fear learning it is also important to point out that several major efferent pathways exit the amygdala (Krettek and Price, 1977a, b; 1978a; Leonard and Scott, 1971; Wallace, Magnuson, and Gray, 1992; Rosen, Hitchcock, Sannes, Miserendino, and Davis, 1991). For the most part amygdaloid efferents exit the amygdala through the medial region of the central amygdaloid nucleus however some efferents do exit via the medial and cortical amygdaloid nuclei and the basolateral amygdaloid nucleus (Krettek and Price, 1978a; Rosen, et al., 1991; De Olmos, Alheid, and Beltramino, 1985; Price, Russchen and Amaral, 1987; McDonald, 1991; Kita and Kitai, 1990). Tracing studies indicate that the amygdala projects to numerous cortical, subcortical and mid-brain

regions (Krettek and Price, 1977a,b; 1978a; Price, et al., 1987; De Olmos, et al., 1985; Wallace, et al., 1992; Rosen, et al., 1991; McDonald, 1991; Kita and Kitai, 1990). While the amygdala's efferents are substantial, the amygdala can be best described as having two or perhaps three well-defined efferent projection pathways that journey through different parts of the central nervous system to target numerous brain nuclei.

The first is forward towards cortical and limbic regions (i.e. subcortical) in the forebrain via the rostral ventral amygdalofugal pathway (rVAF) and stria terminalis (ST). Basically, the rVAF and ST pathways constitute the ascending projections arising from the several distinct amygdaloid nuclei. The rostral subcortical/limbic structures receiving innervation from the amygdala include; the BNST, septum, nucleus accumbens, ventral putamen and substantia innominata (Krettek and Price, 1978a; Russchen and Price, 1984; McDonald, 1991). Some of the rostral amygdaloid projections, particularly those emanating from the BLA, have been shown to provide robust innervation of the nucleus accumbens, striatum and prefrontal cortex (Krettek and Price, 1977a; 1978a; Russchen and Price, 1984; McDonald, 1991; Kita and Kitai, 1990). However, as a rule, other amygdaloid nuclei such as the central and basomedial nuclei also innervate some of these forebrain regions (Krettek and Price, 1978a).

The second pathway represents the descending amygdaloid subcortical projection that innervates the hypothalamus and eventually makes its way to several nuclei located in the midbrain, pons, medulla, and spinal cord (Kretteck and Price, 1978a; Hopkins and Holstege, 1978; Wallace, et al., 1992; Rosen, et al., 1991; Post and Mai, 1980; Schwaber, Kapp, Higgins, and Rapp, 1982; Sandrew, Edwards, Poletti and Foote, 1986; Price, et al., 1987). These innervated regions include; the PAG, VTA, SN, and central tegmentum of the midbrain, the locus coeruleus, parabrachial nucleus and the reticularis pontis caudalis (RPC) of the pons, and finally the nucleus of the solitary tract and the dorsal motor nucleus of the rostral medulla (Krettek and Price, 1978a; Hopkins and Holstege, 1978; Price, et al., 1987; Wallace, et al., 1992; Rosen, et al., 1991; Post and Mai, 1980; Schwaber, et al., 1982). One part of this descending efferent pathway is called the ventral amygdalofugal pathway (VAF) whilst the other is called the caudal VAF (cVAF) pathway and a wealth of research indicates this amygdala-based pathway forms the major output neural circuitry that makes it possible for animals to express autonomic and behavioural responses that are

indicative of conditioned fear or a central fear state (Rosen, et al., 1991; Hitchcock and Davis, 1991; Hitchcock, Sananes, and Davis, 1989; Iwata, Chida, and LeDoux, 1987; Kapp, Gallagher, Underwood, McNall, and Whitehorn, 1982; Rosen and Davis, 1988a,b; for a review see Davis, 1992a; LeDoux, 1993; Fendt and Fanselow, 1999).

The third amygdaloid efferent projection is to temporal, parietal and prefrontal cortical areas. Many amygdaloid nuclei that receive sensory input from such temporal/parietal regions as the perirhinal, entorhinal and insula have been observed sending discrete efferents directly back to the neurons found in these cortical structures (McDonald, 1998). In addition, the basolateral amygdala (BLA) sends extensive efferents to the prefrontal cortex (Krettek and Price, 1977a; Kita and Kitai, 1990; McDonald, 1991) and much of the amygdala in turn receives reciprocal innervation from the prefrontal cortical region and parts of the basal forebrain (Beckstead, 1979; Ottersen, 1982; McDonald, 1998; Kelly, Domesic, and Nauta, 1982; Ottersen, 1980). The amygdaloidal projections back to cortical structures may represent a feedback loop whereby amygdala neurons are able to keep in contact with cortical regions that transmit refined sensory information to the lateral and basolateral nuclei of the amygdala.

3.81: Amygdala Projections via the Stria Terminalis and rVAF Pathway to the BNST, Septum, Nucleus Accumbens, and Prefrontal Cortex

In general, anatomical tracing work by Krettek and Price (1978a) and various others (Russchen and Price, 1984; McDonald, 1991; Kita and Kitai, 1990) indicates that various amygdaloid nuclei project to the BNST, nucleus accumbens, and septum. More specifically, Krettek and Price (1978a) observed that basolateral (BL) and central (Ce) amygdaloid nuclei innervate the lateral BNST, whilst the basomedial amygdala (BMA) target the adjacent portions of both the medial and lateral BNST. The posterior and medial cortical amygdaloid nuclei and the AHA also innervate the medial division of the BNST and the septum (Krettek and Price, 1978a). The projection of the posterior cortical amygdaloid nucleus to the septum can be described as light but the AHA innervation of the ventral lateral septum is quite robust (Krettek and Price, 1978a).

With reference to the nucleus accumbens, anterograde tracer injected into the BLA was observed to produce a thick layer of silver grains that were concentrated in the ventral

striatum, nucleus accumbens and parts of the olfactory tubercle (Krettek and Price, 1978a). Specifically, administration of anterograde tracer into the anterior regions of the BLA produced labelling in the lateral and anterior nucleus accumbens. In contrast, similar application of anterograde tracer to the posterior BLA only produced labelling in the posterior and medial aspects of the nucleus accumbens (Krettek and Price, 1978a). When the BMA was infused with the anterograde tracer Krettek and Price (1978a) demonstrated that ventral aspects of the nucleus accumbens receive input from this amygdaloid region. Generally speaking, the early work by Krettek and Price (1978) is consistent with later tracing studies using PHA-L and more advanced tracing techniques (Russchen and Price, 1984; McDonald, 1991). In essence, these tracing studies all seem to indicate that the BLA and BMA innervate the nucleus accumbens, ventral striatum and basal forebrain regions (see Figure 6). The amygdaloid innervation of the nucleus accumbens and striatum may be very important as these regions may form the neural circuitry necessary for initiating voluntary motor responses during fear/aversive-motivated learning (Salamone, 1994; Killcross, et al., 1997; Cardinal, Parkinson, Hall and Everitt, 2002).

Tracing studies also demonstrate that the BLA sends efferents to the prefrontal cortex and other nearby regions (Krettek and Price, 1977a; McDonald, 1991; Kita and Kitai, 1990). On the whole, the BLA provides a rich innervation to the prefrontal and entorhinal cortical areas including the olfactory tubercles (Krettek and Price, 1977b; 1978a; Kita and Kitai, 1990). As was mentioned earlier, the prefrontal cortex (i.e. medial and orbital areas) projects back to the central and basolateral amygdaloid nuclear groups (Beckstead, 1979; Ottersen, 1982; Carmichael and Price, 1995; McDonald, 1998) and both the amygdala and prefrontal cortex receive an extensive dopaminergic innervation from the A10 and A9 dopamine cell groups of the VTA and SN (Fallon and Moore, 1978; Fallon, Koziell and Moore, 1978; Swanson, 1982; Oades and Halliday, 1987; Loughlin and Fallon, 1983; Simon, Le Moal, and Calas, 1979). This is important, since mesoamygdaloid and mesocorticolimbic dopamine systems are believed to be necessary for facilitating conditioned fear learning and expression (Nader and LeDoux, 1999a,b; Greba and Kokkinidis, 2000; Borowski and Kokkinidis, 1996; Waddington-Lamont and Kokkinidis, 1998; Guarraci, Frohardt and Kapp, 1999; Salamone, 1994) and stress responses (Deutch, Tam, and Roth, 1985; Morrow, Elsworth and Roth, 1996; Inglis and Moghaddam, 1999).

This is particularly relevant as high mesocorticolimbic dopamine levels and an over-active dopamine system have been implicated in the emotional and behavioural psychopathology associated with schizophrenia (Matthysse, 1978; Stevens, 1979; Lewis, 1980; Reynolds, 1983; 1992; Johnson, Aylward, and Totterdell, 1994; Yang, Seamans, and Gorelova, 1999).

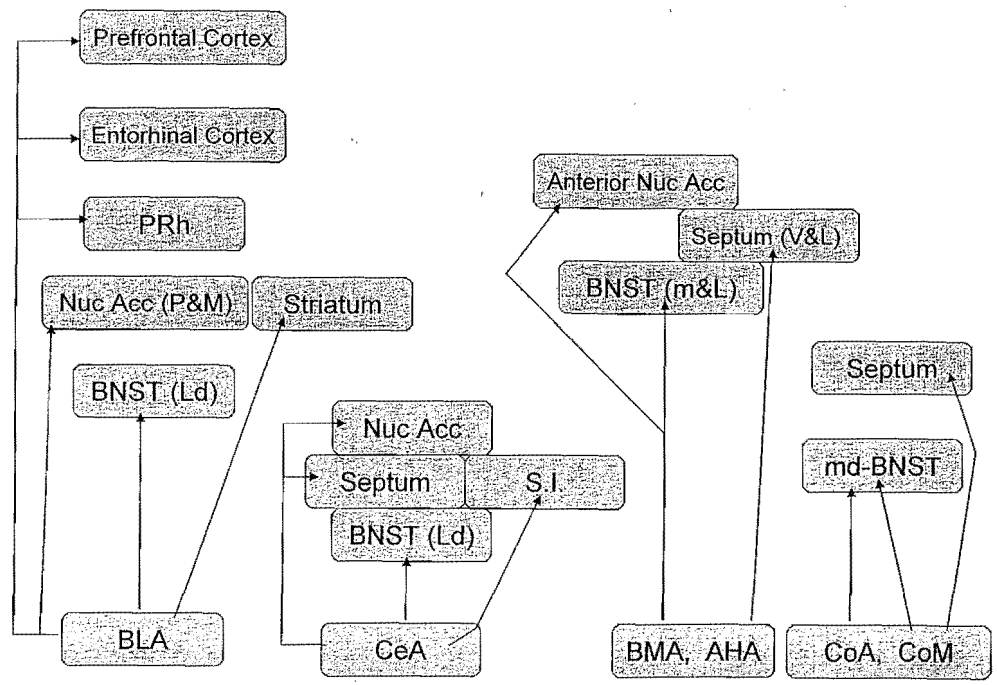

Figure 6. The figure presented above provides a simple depiction of ascending amygdaloidal projections to some cortical and limbic brain regions. On the left of the figure the BLA can be seen projecting to the lateral and dorsal divisions of the bed nucleus of the stria terminalis [BNST(Ld)], the striatum, the posterior and medial nucleus accumbens [Nuc Acc (P&M)], the perirhinal cortex (PRh), the entorhinal cortex and the prefrontal cortex. The central nucleus of the amygdala (CeA) innervates the BNST(Ld), the substantia innominata (S.I.), the Septum and the Nuc Acc (middle of figure). Further to the right, the basomedial amygdala (BMA) is shown projecting to the medial and lateral divisions of the BNST [BNST (m&L)] and the anterior Nuc Acc, whereas the amygdalohippocampal area (AHA) innervates the ventral and lateral regions of the Septum. Finally, on the extreme right of Figure 6, the anterior cortical amygdaloid nucleus (CoA) can be seen projecting to the medial and dorsal BNST (md-BNST), whilst the medial cortical amygdaloid nucleus (CoM) targets the md-BNST and the Septum.

3.82: Amygdala Projections to the Thalamus, Hypothalamus, Midbrain, Pons, and Medulla via the VAF and caudal VAF pathways

Research garnered from anterograde and retrograde tracing studies has demonstrated that efferent fibres from the central and medial nuclei of the amygdala innervate the dorsomedial and midline thalamic nuclei, and the caudal aspects of the centromedial nucleus located just lateral to the ventral posteromedial nucleus of the thalamus (Price and Amaral, 1981; Aggleton and Miskin, 1984; Krettek and Price, 1977a). As has already been mentioned, the thalamic innervation of the amygdala is quite extensive and the reciprocal connectivity between these two regions undoubtedly plays an important role in facilitating conditioned fear learning. This notion is further supported by electrophysiological research demonstrating that inhibition of central amygdaloid nucleus neurons with muscimol significantly reduces the activity level and firing pattern of lateral geniculate thalamic neurons in rabbits exposed to fear-arousing conditioned stimuli (Cain, Kapp, and Puryear, 2002).

Information reaching the hypothalamus from the amygdala is channelled by the VAF and cVAF pathways. The former, arises primarily from neurons located in the central and basolateral amygdaloid nuclei, but other amygdala regions also project to the hypothalamus (Krettek and Price, 1978a; Gray, Carney, and Magnuson, 1989; Rosen, et al., 1991; Post and Mai, 1980). The latter (i.e. cVAF) pathway is made up of efferents emanating out of the rostral portions of the central nucleus of the amygdala (Krettek and Price, 1978a,; Rosen, et al., 1991). The cVAF pathway innervates parts of the lateral hypothalamus, however this amygdaloid-based pathway mainly projects directly to several brain nuclei located in the midbrain, pons and medulla (Krettek and Price, 1978a; Rosen, et al., 1991; Wallace, et al., 1992; Post and Mai, 1980; Schwaber, et al., 1982). Thus, one portion of the VAF pathway from the amygdala projects to the various hypothalamic nuclei and does not appear to journey beyond this structure. In contrast, the other pathway, a part of which is referred to as the cVAF pathway, passes through the caudal portions of the lateral hypothalamus and eventually makes its way to midbrain and brain-stem regions that regulate autonomic and reflexive responses (Rosen, et al., 1991; Krettek and Price, 1978a; Wallace, et al., 1992; Hitchcock and Davis, 1991; Hitchcock, et al., 1989; LeDoux, Iwata, Cicchetti and Reis, 1988).

3.83: Amygdaloid Efferents Reaching the Hypothalamus via the VAF Pathway

Anatomical studies by various researchers have mapped the path taken by amygdaloid efferents as they travel to the hypothalamus via the VAF pathway (Leonard and Scott, 1971; McBride and Sutin, 1977; Krettek and Price, 1978a; Price and Amaral, 1981; Gray, et al., 1989; Rosen, et al., 1991). In general, most amygdaloid nuclei project to the ventromedial and premammillary nuclei of the medial hypothalamus but there is also a very robust projection by the CeA and BLA to the lateral hypothalamus (Krettek and Price, 1978a). More specifically, fibres originating in the medial and posterior aspects of the cortical amygdaloid nuclei and in the anterior amygdaloid area (AAA) terminate in the shell and core region of the ventromedial hypothalamic nuclei (Krettek and Price, 1978a). In addition, cortical and medial amygdaloid nuclei have also been reported to innervate the supraoptic, paraventricular, and rostral parvicellular aspects of the paraventricular nucleus of the hypothalamus (Gray, et al., 1989; Price, Slotnick and Revial, 1991). Also, Krettek and Price (1978a) noted that the medial and basomedial amygdaloid nuclei send quite extensive projections to the ventromedial and dorsomedial areas of the hypothalamus.

Furthermore, PHA-L tracing by Gray and colleagues (1989) demonstrated that the medial amygdaloid nucleus tends to innervate the rostral parvicellular parts of the paraventricular nucleus of the hypothalamus, whereas the central nucleus of the amygdala primarily targets the lateral and medial portions of the paraventricular hypothalamic nucleus. In addition to this finding, it has been demonstrated that the central nucleus of the amygdala and the basolateral amygdaloid nucleus provide the lateral hypothalamus with a very dense labelling of fibres (Krettek and Price, 1978a; Rosen, et al., 1991). However it should be pointed out that the projections arising from the central amygdaloid nucleus produce very dense labelling in the lateral hypothalamus, whilst those emanating from the BLA cause significantly lighter labelling in this hypothalamic region (Krettek and Price, 1978a). It is also important to note that the BMA and the cortical amygdaloid nuclear groups produce no labelling in the lateral hypothalamus indicating that very few efferents from these areas reach the lateral hypothalamus (Krettek and Price, 1978a). Finally, it should be mentioned that the hypothalamus sends extensive projections back to the central amygdaloid nucleus, the medial and basomedial amygdaloid nuclei, and the accessory basal

nucleus of the amygdala (Ottersen, 1980). As will be seen later on, this closely intertwined amygdalo-hypothalamic neurocircuitry is most likely involved in regulating neuroendocrine functioning during times of extreme stress and fear.

3.84: Amygdaloid Efferents travelling to the Midbrain, Pons, and Medulla via the Caudal VAF Pathway: A Vital Amygdala-based System involved in the Expression of Conditioned Fear

As was noted above, the central and basolateral amygdaloid nuclei innervate the lateral hypothalamus (see Figure 7). In a region just caudal to the lateral hypothalamus and near the subthalamic nucleus, efferents belonging to the central amygdaloid nucleus which form the cVAF pathway begin their descent towards the various nuclei located in the midbrain, pons, medulla and spinal cord (Krettek and Price, 1978a; Hopkins and Holstege, 1978; Veening, Swanson, and Swachenko, 1984; Wallace, et al., 1992; Rosen, 1991; Schwaber, et al., 1982; Sandrew, et al., 1986; Price, et al., 1987). At the level of the midbrain, the cVAF pathway travels through the SN and portions of the VTA (Wallace, et al., 1992; Krettek, 1978; Veening, et al., 1984; Rosen, et al., 1991; Simon, LeMoal, and Calas, 1979). Some of these amygdaloid fibres terminate in the A9 and A10 group of the SN and VTA where a number of tyrosine hydroxylase immunoreactive cells have been observed (Wallace, et al., 1992).

Caudal to the SN the central amygdaloid efferents contained within the cVAF pathway move dorsally and medially as they course through the central tegmental field finally coming into contact with the lateral and ventrolateral portions of the PAG (Krettek and Price, 1978a; Hopkins and Holstege, 1978; Rosen, et al., 1991; Wallace, et al., 1992). The ventrolateral region of the PAG is innervated by these central amygdaloid efferents at the caudal level of the midbrain near the point where the rostral pons starts to become prominent (Rosen, et al., 1991; Wallace, et al., 1992; Krettek and Price, 1978a). Once in the pons, central amygdala efferents travel to the medial and lateral parabrachial nuclear regions where many amygdaloid fibres have been shown to terminate (Wallace, et al., 1992; Hopkins and Holstege, 1978; Amaral, Price, Pitkänen and Carmichael, 1992). While in the pons region, the central amygdaloid efferents belonging to the cVAF fibre system also innervate the locus coeruleus (Wallace, et al., 1992; Cedarbaum and Aghajanian, 1978;

Foote, Bloom and Aston-Jones, 1978) and the sensory and motor nuclei of the trigeminal and facial nerve (Wallace, et al., 1992; Hopkins and Holstege, 1978). Additionally, some of the central amygdaloid nucleus axons terminate in the gigantocellular reticular nucleus and the raphe nucleus which includes the raphe magnus and the raphe pallidus nucleus located at the level of the caudal pons (Wallace, et al., 1992; Hopkins and Holstege, 1978).

Perhaps the most important amygdala projection as far as this thesis is concerned deals with the cVAF pathway that carries central amygdaloid nucleus efferents to the reticularis pontis caudalis nucleus (RPC). Experimental work by Michael Davis and his many co-workers has established that conditioned fear influences the acoustic startle response at the RPC (see Rosen and Davis, 1988a, b, Rosen and Davis, 1990; Lee, López, Meloni, and Davis, 1996b; Davis, 1992a) and that the central nucleus of the amygdala and the cVAF pathway is important for the expression of fear-potentiated startle (FPS) and shock sensitised startle (Hitchcock and Davis, 1991; Hitchcock, et al., 1989). Moreover, the auditory startle pathway which contains giant cochlear root neurons located in the ventral cochlear nucleus projecting directly to the ventrolateral RPC seems to be vital for mediating the acoustic startle reflexive response (López, et al., 1996b; also see Davis, 1992a; 1997; 2000; Koch, 1999). Rosen and associates (1991) and various others (Hopkins and Holstege, 1978; Krettek and Price, 1978a; Post and Mai, 1980; Wallace, et al., 1992) have demonstrated that central amygdala nucleus fibres innervate the RPC at the level of the caudal pons in an area slightly dorsal to the superior olives (see Rosen, et al., 1991; for a review see Davis, 1992a; 2000; Koch, 1999). Finally, at the level of the medulla, central amygdaloid nucleus efferents innervate the nucleus of the solitary tract and the dorsal motor nucleus of the vagus with some fibres entering the cervical levels of the spinal cord (Wallace, et al., 1992; Danielsen, Magnuson, and Gray, 1989; Hopkins and Holstege, 1978; Schwaber, et al., 1982; Veening, et al., 1984). Comprehension of the efferent projections of the central nucleus of the amygdala to the hypothalamus, midbrain, pons and medulla will be quite valuable to the reader as the next chapter begins to discuss how the amygdala and its efferent projections are involved in influencing emotional responses and expressions of fear and anxiety. In addition, having a working knowledge of some of the ascending amygdaloidal projections to the BNST, septum, striatum, nucleus accumbens and prefrontal

cortex will also be helpful when the amygdala's involvement in fear, anxiety, stress and schizophrenia is discussed in the next chapter.

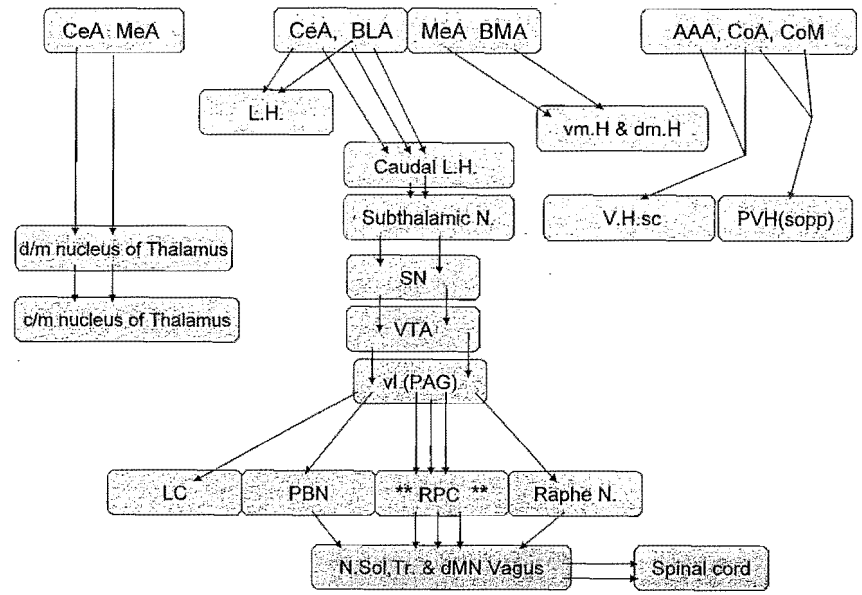

Figure 7 presented above depicts some of the key descending amygdaloid projections to sub-cortical and midbrain structures. On the left of the figure the central nucleus and medial nuclei of the amygdala (CeA and MeA) project to the dorsal and midline thalamic nuclei and the centromedial nucleus of the thalamus. In the middle of Figure 7, CeA, and BLA send projections to the lateral hypothalamus (L.H.). Most important are the projections (depicted by 3 arrows) innervating the caudal L.H. close to the sub-thalamic nucleus. This pathway represents the caudal ventral amygdalofugal pathway to the midbrain and pons. This direct pathway to the reticularis pontis caudalis (RPC) is important for FPS expression. Basically, efferents from the medial CeA and parts of the BLA travel to the substantia nigra (SN), the ventral tegmental area (VTA) and the ventrolateral periaqueductal gray (vlPAG). Some synaptic contacts are made in these midbrain areas. However, a majority of CeA and BLA efferents reach the RPC which is an important nucleus for mediating FPS in rats. Within the pons CeA and BLA efferents target the locus coeruleus (LC), the parabrachial nucleus (PBN) and the Raphe nucleus. Efferents from the CeA and BLA reach the nucleus of the solitary tract (N.Sol, Tr.) and the dorsal motor nucleus of the vagus (dMN vagus) and then enter the spinal cord. Further to the right, the medial nucleus of the amygdala (MeA) and the BMA innervate the ventromedial and dorsomedial hypothalamic nuclei (vm.H & dm.H). Finally, the anterior amygdaloid area (AAA) and the anterior cortical nucleus of the amygdala (CoA) innervate the shell and core of the ventromedial hypothalamic nuclei, whilst the CoA and the medial cortical nucleus of the amygdala (CoM) target the supraoptic, paraventricular, and parvicellular aspects of the paraventricular nucleus of the hypothalamus (PVH soop).

Chapter 4

The Role of the Amygdala in Emotion and Fear

The amygdala's anatomical and cellular diversity coupled with its vast array of afferent and efferent connections with other brain regions places this limbic structure in an excellent position to assess the biological and emotional significance of both appetitive and aversive motivational stimuli. Experimental manipulation of the amygdala allows scientists to examine the neuroanatomical substrates and neurochemical processes underlying fear acquisition, expression and reinstatement. For example, many of the neural substrates innervated by the central nucleus of the amygdala are involved in mediating specific autonomic, behavioural, and hormonal changes that are either indicative of, or associated with emotionality (i.e. fear, and anxiety). Thus, bilateral lesioning of the amygdaloid central nucleus generally blunts almost all expressions of emotionality, whereas lesioning of the brain structure innervated by amygdaloid efferents attenuates selective expressions of emotion (for a review see Davis, 1992a,b,c; 1993; 1997; LeDoux, 1992; 1993; 2000; Fendt and Fanselow, 1999; Koch, 1999).

In contrast, research indicates that the lateral and/or basolateral amygdaloid complex appears to be involved in the acquisition of conditioned fear and in the retrieval and permanent storage of fear-memories (Davis, 1992a,b; 1997; LeDoux, 1992; LeDoux, 2000; Fendt and Fanselow, 1999; Koch, 1999; Davis, 2000 Maren, 1999; 2001). Although there is some debate regarding the BLA's role in fear memory storage and consolidation (see Cahill and McGaugh, 1998; Cahill, Weinberger, Roozendaal, and MaGaugh, 1999; Weinberger, 1998 versus Davis, 1997; 2000 Fanselow and LeDoux, 1999; LeDoux, 2000; 2002; Maren, 1999; 2001) it is generally accepted that the amygdala plays a crucial role in fear learning and expression and in the innate and unconditioned responses that are indicative of a central fear state (for reviews see Davis, 1992a,b,c; 1997; 2000; LeDoux, 1992; 1993; 2000; Fendt and Fanselow, 1999; Koch, 1999; Maren, 2001).

4.1: The Amygdala's Participation in Unconditioned Fear Responding, Innate Fear Expression, Stress, and Anxiety-like Behaviour

In general, a central fear state produces a number of physiological and behavioural effects. These effects include, learned responses and modified reflexes generated by exposure to a conditioned stimulus following extensive Pavlovian fear conditioning, while other fear manifestations can be attributed to the acute and sudden exposure to either innate or aversive environmental stimuli (i.e. predator odour, foot shock, high levels of illumination), (see Blanchard R.J. and Blanchard D.C., 1969; 1971; 1989; Cattarelli and Chanel, 1979; Davis, 1992; 1997; LeDoux, 1992; 2000; Burwash, Tobin, Woolhouse and Sullivan, 1998; Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999; Koch, 1999; Maren, 1999a; Perrot-Sinal, Ossenkopp and Kavaliers, 1999; Morrow, Redmond, Roth and Elsworth, 2000a; Morrow, Roth, and Elsworth, 2000b; Zamgrossi Jr. and File, 1992; Wallace and Rosen, 2000; 2001; McGregor, Schrama, Ambernoon, and Dielenberg, 2002). The former is referred to as conditioned fear which has a learning component, whereas the latter is referred to as unconditioned fear since it is not associated with a specific cue, but rather is produced by direct acute exposure to an aversive stimuli.

Fear, whether it be conditioned or unconditioned is a species specific motivational state that occurs when an organism is exposed to natural predators, threatening events, or stressful (i.e. anxiogenic) environmental stimuli (Bolles, 1970; Fanselow and Lester, 1988 in Bolles and Beecher; Fox and Sorenson, 1994). This motivational state prepares the organism for action and is often characterized by several significant autonomic and behavioural changes that are indicative of a central fear state. For example, innate and/or unconditioned fear produced by exposure to predator odours will cause rats to initiate defensive freezing and avoidance behaviours and will generally lead to marked alterations in dopamine (DA) metabolic activity and neuroendocrine function (Cattarelli and Chanel, 1979; Blanchard R.J., and Blanchard D.C., 1989; Burwash, et al., 1998; Perrot, et al., 1999; Morrow, et al., 2000a, b; Wallace and Rosen 2000). In most vertebrates, the behavioural responses typically associated with this central fear-induced motivational state includes; hypervigilence, defensive freezing, flight/escape behaviours, increased acoustic startle responding, aggressive attack, crouching, urination and defecation (Kaada, 1967; Blanchard and Blanchard, 1971; Blanchard and Blanchard, 1972; Bolles, 1970; Blanchard, Fukunaga

and Blanchard, 1976; Cattarelli and Chanel, 1979; Fanselow and Bolles, 1982; Fanselow, 1984; Fanselow, 1986; Blanchard and Blanchard, 1989; Fanselow and Lester, 1988; Davis, 1992; 1997; LeDoux, 1992; Zangrossi Jr. and File, 1992; Roozendaal, Koolhaas and Bohus, 1991a; 1991b; McGregor, et al., 2002) as well as active defensive burying and avoidance of fearful objects (Blanchard and Blanchard, 1969; 1970a b; 1989; Pinel and Treit, 1978; Bolles, 1970; Roozendaal, Koolhaas and Bohus, 1991b; Burwash, et al., 1998).

These defensive behaviours are generally accompanied by autonomic nervous system arousal, marked elevations in neuroendocrine activity, and increased corticosterone levels in blood plasma, and bradycardia (Roozendaal, Koolhaas and Bohus, 1991a b; Morrow, et al., 2000a). For example, acute unsignalled shock exposure elicits defensive freezing, increases bradycardia and arterial blood pressure, elevates blood plasma levels of epinephrine, norepinephrine and corticosterone and sensitises the acoustic startle reflex (LeDoux, et al., 1988; Roozendaal, Koolhaas and Bohus, 1991a; Davis, 1989a). Lesioning of the central amygdaloid nucleus disrupts bradycardia and behavioural immobility normally produced by acute shock exposure (Roozendaal, Koolhaas, and Bohus, 1990; Roozendaal, et al., 1991b), whilst NMDA lesions of either the anterior BLA or electrolytic lesions of the central nucleus of the amygdala block the shock sensitisation of startle (Hitchcock, Sananes, and Davis, 1989; Sananes and Davis, 1992). Moreover, electrolytic lesions of the central amygdaloid complex significantly reduce plasma blood levels of prolactin, epinephrine, norepinephrine, and corticosterone (Roozendaal, et al., 1991a). Similarly, the heightened levels of plasma corticosterone induced by restraint stress reportedly falls off sharply after the central nucleus of the amygdala is lesioned (Beaulieu, DiPaolo, and Cote, 1987; Beaulieu, DiPaolo and Barden, 1986).

It has been demonstrated experimentally that the bradycardia stress response which typically follows acute shock exposure is likely mediated by a peptidergic monosynaptic pathway beginning with neurons in the central nucleus of the amygdala projecting to the dorsomedial medulla and specifically targeting the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, and the nucleus ambiguus (Danielsen and Magnuson, 1989; Hopkins and Holstege, 1978; Krettek and Price, 1978a; Rogers and Fryman, 1988; Schwaber, Kapp, and Higgins, 1980; Schwaber, Kapp, Higgins and Rapp, 1982; Higgins and Schwaber, 1983). Evidence supporting the involvement of the amygdala and this

pathway comes from many experimental investigations that have consistently demonstrated that lesioning of the central nucleus of the amygdala impairs bradycardia and reduces behavioural immobility evoked by electrical shock or stressful situations (Kapp, Gallagher, Underwood, McNall and Whitehorn, 1982; Roozendaal, Koolhaas, and Bohus, 1990; 1991ab). Further support for the involvement of the amygdala in regulating autonomic and behavioural unconditioned fear responses at the level of the midbrain, pons and medulla (i.e. at the nucleus of the solitary tract and dorsal motor nucleus) comes from electrophysiological studies that have shown that electrical and/or chemical stimulation of the central nucleus of the amygdala produces behavioural immobility, (Applegate, et al., 1983) decreases heart rate and blood pressure, (Reis and Oliphant, 1964; Mogenson and Calaresu, 1973; Applegate, et al., 1983; Gelsema, McKitrick and Calaresu, 1987; Kapp, et al., 1982; Iwata, Chida and LeDoux, 1987; Brown and Gray, 1988) causes immediate and synchronized increases in catecholamine levels in the brain and blood plasma, (Reis and Gunne, 1965; Brown and Gray, 1988; Dietl, 1985) and enhances acoustic startle responding (Rosen and Davis, 1988a,b; Koch and Ebert, 1993).

Indeed, the presence of moderate to heavily labelled axons emanating from the central amygdaloid nucleus and terminating in the adrenergic catecholaminergic and noradrenergic cell groups in the nucleus of the solitary tract, the dorsal motor nucleus of the vagus and various other brainstem regions provides compelling evidence that the central nucleus of the amygdala regulates many of the autonomic and behavioural unconditioned fear responses mentioned above (Wallace, Magnuson and Gray, 1992; Veening, Swanson, and Sawchenko, 1984). For example, research has shown that central amygdaloid efferent pathways passing through but not synapsing in the lateral hypothalamus may be critically involved in modulating unconditioned autonomic reactions to aversive or painful stimuli (LeDoux, Iwata, Cicchetti and Reis, 1988). Thus, ibotenic acid lesions of the lateral hypothalamus failed to block the unconditioned changes in mean arterial blood pressure evoked by footshock (LeDoux, et al., 1988). In fact, tracing studies employing PHA-L in combination with glucose-oxidase immunocytochemistry techniques have shown that these central amygdaloid nucleus efferents not only innervate the lateral hypothalamus but also journey through the hypothalamus via the medial forebrain bundle where they eventually form the dorsal bundle that enters and travels through the midbrain region in the dorsal and

ventral portions of the central tegmental tract (Hopkins and Holstege, 1978; Krettek and Price, 1978a; Wallace, et al., 1992; Cassell and Gray, 1989; Wallace, Magnuson and Gray, 1989; Van Der Kooy, Koda, McGinty, Gerfen and Bloom, 1984).

At the more rostral levels of the midbrain, labelled amygdaloid fibres innervate the SN and VTA, two brain structures known to contain numerous DA neurons (Wallace, et al., 1992; Swanson, 1982; Oades and Halliday, 1987) while at more caudal levels amygdaloid axons exit out from the dorsomedial aspects of the dorsal tegmental bundle to innervate the ventrolateral parts of the periaqueductal grey (Wallace, et al., 1992; Cassell and Gray, 1989; Van Der Kooy, et al., 1984; also see Rosen, Hitchcock, Sananes, Miserendino and Davis, 1991). It is possible that projections from the central nucleus of the amygdala to the VTA play a prominent role in mediating the stress-induced elevation in DA metabolites observed in the prefrontal cortex (Fadda, Argiolas, Melis, Tssari, Onali and Gessa, 1978; Herman, Guillonneau, Dantzer and Scatton, 1982; Claustre, Rivy, Dennis and Scatton, 1986; Goldstein, Rasmussson, Bunney and Roth, 1996; Deutch, Tam and Roth, 1985). This seems plausible as exposure to mild stress, footshock, and conditioned fear cues selectively enhances DA metabolism in the amygdala, VTA and prefrontal cortex (Thierry, Tassin, Blanc, and Glowinski, 1976; Fadda, et al., 1978; Herman, et al., 1982; Ida, Tsuda, Seyoshi, Shirao and Tanaka, 1989; Coco, Kuhn, Ely, and Kilts, 1992; Deutch, Tam and Roth, 1985; Deutch, Lee, Gillham, Cameron, Goldstein, and Iadarola, 1991; Inglis and Moghaddam, 1999). Additionally, the fear/stress-induced increase in DA metabolites in the prefrontal cortex can be attenuated by benzodiazepine treatment (Fadda, et al., 1978; Lavielle, Tassin, Thierry, Blanc, Herve, Berthelemy and Glowinski, 1978; Ida, et al., 1989; Coco, et al., 1992), destruction of noradrenergic fibres innervating the VTA (Herve, Blanc, Glowinski, and Tassin, 1982), or lesioning of the central amygdaloid nucleus (Davis, Hitchcock, Bowers, Berridge, Melia, and Roth, 1994; Goldstein, et al., 1996).

Furthermore, electrical stimulation of the central nucleus of the amygdala has been shown to alter the firing rates of VTA neurons (Maeda and Mogenson, 1981) and electrical stimulation of the VTA significantly increases startle responsiveness in rats (Borowski and Kokkinidis, 1996). Also, electrical stimulation of the VTA and SN DA neurons projecting to the BLA (Swanson, 1982; Loughlin and Fallon, 1983) caused fast firing neurons in the basolateral amygdaloid nucleus to increase their firing rates even more, whereas slow firing

neurons in this amygdaloid region exhibited a marked decrease in activity (Rozenkranz and Grace, 1999). In a related manner, both VTA and amygdaloid kindling significantly increase expressions of fear in laboratory animals (Rosen, Hammerman, Sitcoske, Glowa and Schulkin, 1996; Stevens and Livermore, 1978) and VTA dopaminergic neurons firing patterns are altered during fear conditioning (Guarraci and Kapp, 1999).

In addition, intra-VTA infusion of either the D_2 receptor agonist quinpirole hydrochloride or the GABAergic agonist muscimol, two drugs that typically inhibit DA neurons and alter DA release (Westerink, Kwint, and deVries, 1996; White and Wang, 1984) have been shown to block both the shock sensitisation of startle and FPS in laboratory rats (Gifkins, Greba and Kokkinidis, 2002; Munro and Kokkinidis, 1997; Borowski and Kokkinidis, 1996). These findings seem to indicate that mesoamygdaloid neurodynamics may play a prominent role in mediating conditioned fear expression. As a matter of fact, there seems to be a great deal of support for this claim as it has recently been shown that blockade of DA D_1 receptors in the amygdala impairs both the acquisition and expression of conditioned fear in both the defensive freezing and FPS paradigms (Guarraci and Kapp, 1999; Guarraci, Frohardt and Kapp, 1999; Waddington-Lamont and Kokkinidis, 1998; Greba and Kokkinidis 2000; Nader and LeDoux, 1999b).

At even more caudal levels, the labelled fibres from the central nucleus of the amygdala innervate the pontine parabrachial nucleus, reticularis pontis caudalis, locus coeruleus and medulla specifically terminating in the dorsal motor nucleus of the vagus, the nucleus of the solitary tract or the ventrolateral medulla (Wallace, et al., 1992; Wallace et al., 1989; Hopkins and Holstege, 1978; also see Krettek and Price, 1978a; Rosen, et al., 1991). Given this anatomical arrangement it is likely that amygdala activation can influence unconditioned fear effects by its innervation of various midbrain and brainstem nuclei. For example, electrical and chemical stimulation of the CeA or PAG enhances unconditioned defensive freezing, increases acoustic startle amplitudes, produces flight behaviour and fear-like responses and elicits aggressive defensive responding in laboratory animals (Applegate, Kapp, Underwood, and McNall, 1983; Bandler and Carrive, 1988; Bandler and Depaulis; 1988; Carrive, 1993; Bandler, 1982; Di Scala, Schmitt and Karli, 1984; Fardin , Oliveras and Besson, 1984; Fernandez de Molina, and Hunsperger, 1962; Rosen and Davis, 1988; Boulis and Davis, 1989; Koch, 1993; Koch and Ebert, 1993; Behbehani, 1995).

Also, the central amygdaloid nucleus innervation of the caudal periaqueductal gray (PAG) area is crucial for mediating both conditioned and unconditioned defensive freezing following exposure to either an acute footshock or a conditioned fear stimulus (Roozendaal, et al., 1991a; LeDoux, Iwata, Cicchetti and Reis, 1988; Fanselow, 1991; in Depaulis and Bandler; Fanselow, DeCola, De Oca and Landeira-Fernandez, 1995; Beitz, 1982; Beitz, 1995). It is important to note that freezing behaviour is generally disrupted following electrolytic or chemical lesions to either the CeA or caudal PAG (Roozendaal, et al., 1991a, b; LeDoux, et al., 1988; Fanselow, et al., 1995; Blanchard, D.C., Williams, Lee, et al., 1981; Fanselow, 1991 in Depaulis and Bandler pp 151-173). Furthermore, lesions of the central nucleus of the amygdala, its efferent pathway to the reticularis pontis caudalis, or the PAG block the shock sensitisation of acoustic startle responding in rats (Fendt, Koch and Schnitzler, 1994; Hitchcock, Sanannes, and Davis, 1989; Rosen, et al., 1991).

In stark contrast, electrical or chemical stimulation of the amygdala or its efferent pathway to the reticularis pontis caudalis have been shown to significantly elevate acoustic startle amplitudes (Rosen and Davis, 1988; Boulis and Davis, 1989; Davis, Gendleman D.S., Tischler, and Gendleman P., 1982; Koch, 1993; Koch and Ebert, 1993). Similarly, stimulation of the dorsolateral PAG has been shown to significantly enhance the acoustic startle reflexive response and cause jumping and flight behaviours that are indicative of fear and anxiety (Bandler and Depaulis, 1988; 1991; Behbehani, 1995; Carrive, 1993; Borowoski and Kokkinidis, 1996). These results indicate that direct projections from the central amygdaloid nucleus to the midbrain startle circuit that includes input from neurons in the PAG, may be involved in mediating the sensitizing effects of shock on acoustic startle responding (Hitchcock, Sanannes, and Davis, 1989; Boulis and Davis, 1989; Rosen, et al., 1991; Koch and Ebert, 1993; Koch, 1993; Fendt, et al., 1994; Davis, et al., 1982b; Davis, Gendleman, D.S., Tischler, and Gendleman, P., 1982a).

However it is debatable whether or not shock sensitised startle truly represents an unconditioned fear response given that it develops gradually (Davis, 1989a) and has been linked to contextual fear conditioning (see Richardson and Elsayed, 1998; Pilz, 1996; Kiernan, Westbrook and Cranney, 1995). Similar arguments have also been made about whether defensive freezing after unsignalled shock exposure truly represents an unconditioned fear effect or whether it represents a conditioned behavioural fear response

that develops after contextual cues are associated with unsignalled footshock (Fanselow, 1980; 1982; Fanselow and Bolles, 1982; Fanselow, 1984; Fanselow, 1986). For example, research has demonstrated that defensive freezing does not occur after immediate exposure to unsignalled footshock but rather develops as a function of the time spent in the experimental context prior to footshock exposure (Blanchard, Fukunaga and Blanchard, 1976; Fanselow, 1986).

Fanselow (1986), for instance, found that rats required a minimum of between twenty-seven to eighty-one seconds of contextual exposure prior to unsignalled footshock administration to cause significant defensive freezing, although some freezing behaviour does begin to emerge around nine seconds after aversive footshock administration. Based on these and other results (Fanselow, 1984, Fanselow and Bolles, 1982; Fanselow, 1982), Fanselow (1986) concluded that freezing behaviour that occurs shortly after the termination of the unsignalled footshock is likely a conditioned fear response that is caused by shock associated cues (i.e. the context) and not by a reaction to the shock alone. Thus, according to Fanselow (1986) defensive freezing depends on the activation of a mediator (i.e. fear) which is produced by shock-associated contextual stimuli and not by the shock itself since immediate unsignalled footshock presentations did not result in immediate freezing behaviour. In fact the reported delays in defensive freezing to unsignalled footshock presentation closely parallel the type of delays reported in the shock sensitisation startle paradigm (Fanselow, 1986; Davis, 1989).

Davis (1989) reported delays in the development of shock sensitised acoustic startle responding. This delay in the onset of shock sensitisation led Davis (1989) to suggest that the unconditioned effects produced by the footshock could become associated with a neutral stimulus to facilitate classical conditioning. More recently, Richardson and Elsayed (1998) have revisited Davis's (1989) idea and their research has led to them to contend that shock sensitisation is a form of contextual fear rather than an unconditioned fear effect. Essentially, Richardson and Elsayed (1998) expanded on Davis's (1989) work and used some of it to buttress their arguments and research which indicates that shock sensitisation is a form of contextual fear conditioning. However, it should be emphasized that shock sensitization may develop quicker than originally reported (Gifkins, Greba and Kokkinidis, 2002) and can even occur when unsignalled footshocks are presented in a context that is

distinct to the startle testing apparatus (see Willick and Kokkinidis, 1995; Greba, Gifkins and Kokkinidis, 2001 unpublished results).

Moreover, Gewirtz, McNish, and Davis, (1998) using a repeated training and testing procedure over a 20 day period (10 baseline noise followed by 3 noise-alone and 3 CS + noise test trials then terminating with 2 CS + shock trials) found that only shocked rats showed incremental and significant increases on the 10 baseline noise-alone trials presented before the testing and training trials (3 noise-alone vs. 3 CS + noise and 2 CS + shock). Non-shocked control rats or animals that had received lesions of the BNST prior to training failed to show this long-term shock-sensitization effect (Gewirtz, et al., 1998). According to Gewirtz, et al., (1998) this shock sensitization effect did not appear to be indicative of contextual fear conditioning but rather seemed to be the result of long-term sensitization of startle that develops due to repeated footshock exposure over time. Based on this finding it is tempting to speculate that this type of repeated and prolonged shock exposure may increase stress-induced anxiety and lead to a memory trace of the shock being formed in the amygdala or BNST.

With regards to the defensive freezing results reported by Fanselow (1986; see above) it should be pointed out that unsignalled footshock is a painful stimulus that may produce increased arousal and reactive responses such as jumping and searching for an escape route that are highly incompatible with defensive freezing (Fanselow, 1980; 1982). In addition, it is important to note that interpretations regarding what is defined as conditioned fear or unconditioned fear or when and even how painful stimuli become linked to environmental and/or specific stimuli through Pavlovian conditioning depends to a large degree on whether one uses Wagner's "affective extension sometimes-opponent-process" (AESOP) model or the "perceptual-defensive-recuperative" (PDR) model proposed by Bolles and Fanselow (1980) to make such judgements (for a comparison see Wagner and Brandon, 1989; Bolles and Fanselow, 1980).

Basically, the AESOP model suggests that footshock administration produces biphasic patterns of behaviour characterized by a sudden burst of activity during the shock itself (i.e. the A1 state) and just a few seconds after the shock has been terminated (i.e. the A2 state). The A1 and A2 states are followed by a tranquil period called the inactive state (I) where the animal will initiate defensive freezing and other cautious behaviours that are signs of

fear. According to the AESOP model the processing of the footshock information occurs in the active A2 state or phase where the animal is active after the foot shock has been terminated, thus fear is thought to be directly generated by the footshock (Wagner and Brandon, 1989).

In contrast, the PDR model argues that shock exposure alone does not directly produce an internal fear state but rather activates the fear system indirectly by linking together the anatomically and functionally distinct nociceptive and fear motivational systems through Pavlovian fear conditioning (see Bolles and Fanselow, 1980). Hence, the repeated administration of the UCS (e.g. footshock) in a given context or shortly after the presentation of innocuous cue (e.g. light) during Pavlovian fear training effectively unites pain signalling pathways with those systems that are most endowed to generate fear responding. This is why fear is viewed as a mediator in the PDR model. However, caution needs to be exercised when linking unsignalled shock exposure to contextual conditioning because there still is the possibility that the aversive qualities of the footshock may produce a memory-trace of the aversive event in the amygdala especially when it is well known that this limbic brain region receives and processes nociceptive information (LeDoux, et al., 1987; Bernard and Besson, 1990; Bernard, Huang, and Besson, 1992; Bernard, et al., 1993; Turner and Herkenham, 1991; Barnett, et al., 1995; Shi and Davis, 1997; 1999; Shi and Cassell, 1998a,b).

Also, the observation that the shock sensitization of startle can occur quite rapidly after shock administration (Gifkins, Greba and Kokkinidis, 2002) and even when shocks are presented in a context that is not the same as the one used for startle testing (Willick and Kokkinidis, 1995; Greba, Gifkins and Kokkinidis, 2001; unpublished data) does seem to suggest that the information about the aversive nature of the shock is able, in some instances, to elicit fear responding that could be classified as unconditioned. For example, the fear associated with exposure to numerous aversive inescapable shocks in a given test chamber has been shown to transfer readily to a second testing environment (i.e. shuttle boxes) that was never explicitly associated with shock presentations and this has been shown to occur twenty-four hours after the inescapable shock administration (see Maier, 1990; Maier, Grahn, Kalman, Sutton, Wiertelak and Watkins, 1993). Typically, rats that received inescapable shocks exhibit more fear-like defensive freezing behaviour when

immediately placed in the second test context (i.e. shuttle box) than do rats that have not been exposed to inescapable shocks and it is important to note that lesions of the amygdala have been shown to attenuate this effect (Maier, et al., 1993). Interestingly, amygdala lesions also seem to be quite effective in blocking the shock sensitization of startle produced by out of context footshock administration (Greba, Gifkins and Kokkinidis, 2001; unpublished observation) highlighting the important role played by the amygdala in gating the levels of fear and hypervigilence that occur after exposure to aversive stimuli.

Nevertheless, and despite all the debate, it is quite apparent that the amygdala influences unconditioned fear and stress responses as well as conditioned fear and emotional responding through its efferent projections to various subcortical and brainstem structures that are responsible for initiating specific autonomic or behavioural changes in response to fear arousing stimuli (LeDoux, et al., 1988; Roozendaal, et al., 1991a, b; Davis, 1992a, b, c; 1997; LeDoux, 1992; 2000; Fanselow and LeDoux, 1999a). It is also important to point out that lesions of the amygdala that specifically destroy the central nucleus generally block numerous autonomic and behavioural correlates or expressions of fear all at once, whereas lesions of specific midbrain and brainstem structures only effect specific fear responses, whilst leaving others intact (for a review see Davis, 1992a,b,c; 1997; 2000; LeDoux, 1992; 1993; 2000; Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999). Thus it appears as though the amygdala has a global impact on the expression of fear as lesions or temporary pharmacological inactivation of this limbic structure tend to effect a wide range of fear responses (Davis, 1992a, b, c; Davis, et al., 1993; Davis, 1997; 2000; LeDoux, 1993; Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999).

4.2: Clinical Importance of the Amygdala and the Amygdala's role in Innate Fear and Sensorimotor Gating: Amygdaloid Dysfunction, Klüver-Bucy Syndrome, and the Amygdala's Link to Schizophrenia and Epilepsy

4.21: The Amygdala and Innate Fear

The amygdala has also been shown to be involved in the elicitation of numerous innate fear responses and research indicates that several innate fear responses across a variety of animal species can be successfully blocked by amygdala lesioning (Blanchard and

Blanchard, 1972; Ursin, Jellested and Cabrera, 1981; Downer, 1961; Fox and Sorenson, 1994). For example, lesions carried out on either the cortical amygdaloid or central amygdaloid nucleus of the wild rat greatly diminishes emotionality, flight responses, defensive freezing and defensive attack behaviours (Kemble, Blanchard and Blanchard, 1984; Kemble, Blanchard and Blanchard, 1990). It is important to note that large lesions that cause extensive damage to the central amygdaloid nucleus and encompass large portions of the cortical and medial nuclei, often produce a blunting of emotions and an uncharacteristic tameness in wild rats (Blanchard and Blanchard, 1972). Typically, these lesioned rats will make several audacious contacts with a sedated cat and in some instances, crawl all over the cat, indicating a loss of fear and emotionality that is similar in many respects to the symptomatology and emotional pathology often associated with Klüver-Bucy syndrome in primates (Klüver and Bucy, 1937; Wieskrantz, 1956; Downer, 1961; Blanchard and Blanchard, 1972; Kemble, et al., 1984; 1990; Fox and Sorenson, 1994). Hence, bilateral damage to the amygdala or nearby temporal lobe structures reduces innate fear and dulls emotionality across a variety of animal species (Brown and Schafer, 1888; Klüver and Bucy, 1937; Wieskrantz, 1956; Blanchard and Blanchard, 1972; Horel, Keating and Misantone, 1975; Aggleton and Passingham, 1981; Kemble, et al., 1984; 1990; Fox and Sorenson, 1994) including humans (Green, et al., 1951; Scoville, et al., 1953; Terizian and Dalle Ore, 1953; Sawa, et al., 1954; Narabayashi, et al., 1963; Scott, Young, Calder, Hellawell, Aggleton and Johnson, 1997; Adolphs, Tranel, Damasio and Damasio, 1995).

4.3: The Clinical Importance of the Amygdala

In this regard, it is important to note that Klüver-Bucy-like behavioural and emotional pathology were reported to occur in human clinical populations after amygdaloid and temporal lobe removal was employed as a treatment strategy for hyper-emotionality, epilepsy and extreme forms of fear and anxiety (Obratdor, 1947; Green, et al., 1951; Scoville, et al., 1953; Williams, 1953; Sawa, et al., 1954; Pool, 1954; Terian and Ore, 1955; Narabayashi, et al., 1963). Klüver-Bucy-like emotional dysfunctions have also been reported to occur in patients with unilateral temporal lobe and amygdala damage however the deficits are usually less severe and only in rare cases ever reach full-blown status (Bates and Sturman, 1995; Ghika-Schmid, Assal, DeTribolet, and Regli, 1995). More recent

experiments carried out on patients that have had their amygdala surgically removed or damaged by disease or injury indicate that although these individuals appear quite normal on the surface and have reasonable levels of intelligence, they nevertheless have several difficulties and deficits as far as emotionality is concerned (Aggleton, 1992; Adolphs, et al., 1994; Young, Hellawell, Van De Wal and Johnson, 1995).

Some of the most prominent deficits are; a general inability to recognize, interpret, and judge fearfulness in the facial expression of others, a blunting of emotional affect especially as it relates to fear and social interactions, an inability to reproduce or draw fearful faces from memory and a difficulty in both simple and conditioned discrimination tasks (Jacobson, 1986; Aggleton, 1992; Adolphs, Tranel, Damasio H. and Damasio A., 1994; Adolphs, Tranel, Damasio, H. and Damasio, A.R., 1995; LeBar, LeDoux, Spencer and Phelps, 1995; Brok, et al., 1998; Calder, et al., 1996; Adophs, et al., 1999; Scott, et al., 1997). Furthermore, humans with amygdala damage have difficulties in developing conditioned autonomic responses in reaction to aversive stimuli (Halgren, Babb, Rausch, and Crandall, 1978a) and generally show marked impairments in actually producing conditioned fear responses to a CS that is explicitly paired with the UCS (LeBar, et al., 1995). Also in some circumstances, persons with bilateral damage to the amygdala have been reported to have difficulties in assessing the emotional tone of speech (i.e. prosody) when statements are read to these subjects using an angry or fearful voice (Leonard, Rolls, Wilson, and Baylis, 1995).

These above findings are particularly relevant because the ability to recognize fear expressions in others and to produce innate and/or appropriate fear reactions is essential for survival and the harmonious social interaction in most animal species including monkeys and humans (Darwin, 1965; Ekman, 1982; Kling and Brothers, 1992). For example, amygdala or temporal lobe lesioned monkeys often make inappropriate and atypical advances towards the dominant male monkeys in a troop (Kling, Lancaster and Benitone, 1970; Dick, Meyers and Kling, 1969; Kling and Brothers, 1992). This behaviour often results in the lesioned animal falling victim to numerous ferocious attacks by dominant monkeys and to social isolation that in the end, leads to death (Dicks, Meyers and Kling, 1969; Kling, Lancaster and Benitone, 1970; Meyer and Swett, 1970; Aggleton and Passingham, 1981).

In fact, there seems to be a high degree of similarity between some of the emotional deficits and social inappropriateness exhibited by humans and monkeys with amygdaloid damage and the emotional pathology observed in schizophrenic patients (Aggleton, 1993; Kirkpatrick and Buchanan, 1990). Generally speaking, schizophrenic patients like amygdaloid lesioned humans and primates often exhibit noticeable deficiencies in emotionality that include inappropriate mood swings and emotional affect, social isolation, and difficulty identifying emotional states of significant others (Cramer, Bowen, and O'Neill, 1992; Bellack, Mueser, Wade, Sayers and Morrison, 1992; Aggleton, 1993; Kirkpatrick and Buchanan, 1990). A closer neuroanatomical inspection of schizophrenic patients and individuals suffering from long-term anxiety reveals that these people have clearly defined physiological, cytoarchitectural, and biochemical irregularities in the amygdala as well as in the cingulate, entorhinal, and prefrontal cortical regions that innervate the basolateral amygdaloid complex (Berman, Zec, and Weinberger, 1986; Braff and Geyer, 1989; Bogerts, Leiberman Ashtari, Bilder, Degreef, Lerner, Johns, and Masiear, 1993; Raine, Lencz, Reynolds, Harrison, Sheard, Medley, Renolds and Cooper, 1992; Reynolds, 1992; Arnold, Hyman, van Hoesen, and Damasio, 1991; Yang, Kitamura, Nishino, Shirakawa, and Nakai, 1998).

More specifically, post mortem analysis of schizophrenic brains reveals that the size of the amygdala is greatly reduced or appears shrunken when compared to the amygdala found in normal brains taken from subjects that have no history of psychiatric illness and this effect can not be attributed to the use of medications (e.g. antipsychotics) that are designed to control schizophrenia (Bogerts, Meertz and Schonfeldt-Bausch, 1985; Reynolds, 1992). In addition, the left amygdala of schizophrenic subjects often contains significantly higher concentrations of DA or its metabolite homovanillic acid (Reynolds, 1992; 1983) and DA hyperactivity in the amygdala and medial prefrontal regions (i.e. ventral pallidum and nucleus accumbens) has for a long time now been implicated as one of the causal factors contributing to the behavioural and emotional pathology associated with this disease (Randrup and Munkvad, 1967; Stevens, 1973; Matthysse, 1978; Stevens, 1979; Lewis, 1980; Swerdlow, Braff, Geyer, and Koob, 1986; Geyer, Swerdlow, Mansbach, and Braff, 1990; Wan and Swerdlow, 1993; Wan and Swerdlow, 1997; Haber and Fudge, 1997; Inglis and Moghaddam, 1999; Sedvall and Karlsson, 1999). Moreover, it has been revealed that

greater numbers of DA D_2 receptors are present in individuals afflicted with schizophrenia (Reynolds, Reiderer, Jellinger, and Gabriel, 1981; Mackay, Iversen, Rosser, Spokes, Bird, Arregui, Creese and Snyder, 1982; Seeman, Ulpian, Bergeron, Reiderer, Gabriel, and Reynolds, 1984; Reynolds, 1992).

These findings suggest that amygdaloid dysfunction and deficiencies in sensory information processing produced by abnormalities in regions that project to and receive input from the amygdala most likely contributes to much of the exaggerated anxiety-like behaviours, disturbed thought patterns, and impairments in rapid information processing that typically accompany schizophrenia and severe anxiety disorders (Kirkpatrick and Buchanan, 1990; Braff, Stone, Callaway, Geyer, Glick, and Bali, 1978; Nuecherlein, and Dawson, 1985; Bogerts, et al, 1993; Arnold, et al., 1991; Raine, et al., 1992; Haber and Fudge, 1997). For example, schizophrenia suffers exhibit auditory and visual hallucinations, paranoid ideations, anxiety, and disturbances of thought such as delusions of persecution (Kirkpatrick and Buchanan, 1990). Interestingly, these symptoms closely resemble the fearful feelings and hallucinations that are produced by stimulation of the amygdala and other nearby temporal lobe regions in humans (Chapman, 1954; Heath, Munroe, and Mickle, 1955; Baldwin, 1960, Horwitz, Adams, and Rutikin, 1968; Penfield and Perot, 1963; Halgren, Walter, Cherlow, and Crandall, 1978; Gloor, 1990; 1992). Also, some of the fear manifestations observed in epileptic sufferers during seizure activity that is confined to the amygdala closely resembles the fear and anxiety responses that accompany schizophrenia. This may mean that the emotional pathology witnessed in temporal lobe epilepsy patients and schizophrenia sufferers may be attributed to amygdaloid dysfunction. This view seems logical as either epileptic discharge or electrical stimulation confined to the amygdala produces overwhelming feeling of fear in humans and generates a wide range of autonomic and behavioural response that can be defined as correlates of fear and anxiety (see Gloor, 1990; 1992).

Furthermore, schizophrenic patients have been shown to exhibit profound deficits in sensorimotor gating measured by the pre-pulse inhibition of acoustic startle (Braff, Stone, Callaway, Geyer, Glick and Bali, 1978; Braff and Geyer, 1989; Geyer, Swerdlow, Mansbach and Braff, 1990; Braff, Grillon and Geyer, 1992), a technique that has been widely used in experimental situations to provide a reliable operational measure of

sensorimotor gating in both laboratory animals and human subjects (Braff, et al., 1978; Swerdlow, Braff, Geyer, and Koob, 1986; Braff and Geyer, 1990; Geyer, Swerdlow, Mansbach, and Braff, 1990; Braff, et al., 1992; Swerdlow, Braff, Taaid and Geyer, 1994). This has led some to suggest that dysfunction in the amygdala and anterior temporal or medial prefrontal lobes may be at the centre of many behavioural deficits and emotional disturbances of thought and mood that commonly occur in subjects afflicted with schizophrenia (Braff, et al., 1992; Braff and Geyer, 1990; Swerdlow, Caine, Braff and Geyer, 1991a; Yang, et al., 1998).

Indeed, the link between the amygdala and schizophrenia has been considerably advanced by research demonstrating that neurons in the BLA are involved in regulating the sensorimotor gating of acoustic startle in rats (Wan and Swerdlow, 1997; Fendt, Schwienbacher, and Koch, 2000b). This particular research, has demonstrated that either chemical lesioning of basolateral amygdaloid neurons or antagonizing amygdala NMDA receptors with moderate to high doses (i.e. 1.5 μg and 4.5 μg) of AP5 significantly attenuates the pre-pulse inhibition of acoustic startle responding (Wan and Swerdlow, 1997). Wan and Swerdlow (1997) suggest that the BLA most likely accomplishes this task by altering neuronal activity in the ventral pallidum and nucleus accumbens, two brain regions that have also been shown to regulate the pre-pulse inhibition of acoustic startle (Kodsi and Swerdlow, 1994; Swerdlow, Braff and Geyer, 1990; Wan and Swerdlow, 1993). In this regard, it is noteworthy that ventral pallidal neurons can be activated or inhibited by electrical stimulation of the basolateral amygdaloid complex (Maslowski-Cobuzzi and Napier, 1994) thus making it possible that lesioning of the BLA or blockade of NMDA receptors somehow disrupted glutamatergic input and/or transmission to the ventral pallidal region (Fuller, Russchen and Price, 1987).

With this in mind, it is also worth mentioning that a plethora of research literature indicates that DA receptor activation and increased DA release in the amygdala and prefrontal regions may play a fundamental role in the development and maintenance of schizophrenia (Swerdlow, Geyer, Braff and Koob, 1986; Grace, 1991; Deutch, 1992; Mansbach, Geyer, and Braff, 1986; Swerdlow, Keith, Braff and Geyer, 1991; Caine, Geyer, and Swerdlow, 1995; Wan, Taaid, and Swerdlow, 1996; Haber and Fudge, 1997; Sedvall and Karlsson, 1999). This is especially relevant since psychomotor stimulant drugs like

cocaine and d-amphetamine have been shown to exacerbate the positive symptoms of schizophrenia (Angrist, Rostrsen and Gerson, 1980; Akiyama, Kanzaki, Tsuchida and Ujike, 1994) and DA receptor agonists have been shown to impair sensory gating in rats when administered peripherally, an effect that can be blocked by pre-treatment with DA receptor antagonists and certain classes of antipsychotic drugs (Mansbach, et al, 1986; Swerdlow, et al., 1991; Swerdlow, Zisook and Taaid, 1994; Caine, et al., 1995; Wan, Taaid, and Swerdlow, 1996). In this connection it is important to point out that the amygdala contains numerous DA receptors (Boyson, McGonigle, and Molinoff, 1986; Meador-Woodruff, Mansour, Healy, Kuehn, Zhou, Bunzow, Akil, Civelli and Watson Jr, 1991), is a recipient of dopaminergic input from the VTA (Swanson, 1982; Loughlin and Fallon, 1983; Oades and Halliday, 1987; Kilts, Anderson Ely and Maillman, 1988), and projects to other areas such as the ventral striatum and nucleus accumbens (Price, Russchen and Amaral, 1987; Kelley, Domesick, and Nauta, 1982) that also receive robust dopaminergic innervation from midbrain DA neurons located in the VTA and SN (Swanson, 1982).

Indeed, Swerdlow and colleagues (1986; 1990) have demonstrated that central DA hyperactivity in rats produces abnormalities in acoustic startle responding that closely resemble those found in schizophrenic subjects. For instance, DA infusion into the nucleus accumbens disrupted the prepulse inhibition of startle in rats (Swerdlow, Braff, Masten and Geyer, 1990). Taking this into account, it is possible that an over active mesocorticolimbic DA system could combine with NMDA receptor-mediated events in the amygdalopallidal circuit, to produce abnormalities in sensorimotor gating (Swerdlow, Braff, Geyer, and Koob, 1986a; Mansbach, et al., 1986; Swerdlow, et al., 1991; Caine, et al., 1995; Wan, et al., 1996; Fendt, et al., 2000b). It is quite plausible that these abnormalities could underlie abnormal neurochemical processes that in turn may contribute to the cognitive fragmentation and emotional disturbances observed in schizophrenic patients (Swerdlow, et al., 1986; Mansbach, Geyer, and Braff, 1988; Wan and Swerdlow, 1997). This notion is attractive as there is some indication that fear and stress-induced increases in DA metabolites in the amygdala, and prefrontal regions that include the ventral pallidum and parts of the nucleus accumbens (Herman, et al., 1982; Coco, et al., 1992; Goldstein, Rasmusson, Bunney and Roth, 1996; Scatton, D' Angio, Driscoll and Serrano, 1987; Inglis

and Moghaddam, 1999) may fall under the control of glutamatergic systems (Jedema and Moghaddam, 1994; Takahata and Moghaddam, 1998; Feenstra, Botterblom and van Uum, 1998; Fendt, et al., 2000b).

However, there is some indication that this glutamatergic regulated stress-induced DA release in the prefrontal cortex differs to the mechanism of action controlling the release of DA found in the nucleus accumbens (Feenstra, et al., 1998) and this factor may have an impact on how sensorimotor gating is mediated by the amygdalo-accumbens neurocircuitry. For example, Wan and Swerdlow, (1997) have proposed an amygdalo-accumbens based mechanism to explain how pre-pulse inhibition is regulated via projections from the BLA to the shell and core of the nucleus accumbens. These researchers suggest that some pre-pulse inhibition (PPI) is regulated by presynaptic DA and glutamate synergistic interactions taking place at the core of the nucleus accumbens, whilst other pre-pulse events mediated at the shell of the accumbens may not necessarily be DA dependent (Wan, Geyer, and Swerdlow, 1995a).

The former view seems scientifically valid as intra-accumbens infusions of the D_2 receptor agonist quinpirole has been shown to cause impairments in sensorimotor gating in rats (Wan and Swerdlow, 1993) as has the blockade of NMDA receptors in the BLA (Wan and Swerdlow, 1997; Fendt, et al., 2000b). The effects produced by intra-accumbens quinpirole application and NMDA receptor blockade in the amygdala are consistent with reports that peripheral injections of DA agonists (i.e. quinpirole and apomorphine) and glutamate antagonists (i.e. phencyclidine) generally result in a marked reduction in the PPI of acoustic startle responding (Wan and Swerdlow, 1994; Wan, et al., 1996; Mansbach, et al., 1988; Swerdlow, et al., 1991; Caine, et al., 1995; Bakshi and Geyer, 1995; Bakshi, Swerdlow, and Geyer, 1994) in that they indicate that the PPI of startle seems to be regulated by dopaminergic and glutamatergic-mediated events at the core of the accumbens that rely heavily on the functional integrity of NMDA receptors in the basolateral amygdaloid complex (Wan and Swerdlow, 1997).

The only foreseeable difficulty with this view is that treatment with haloperidol which generally reverses PPI impairments caused by DA agonists administered peripherally or directly into the nucleus accumbens (Mansbach, et al., 1988; Wan and Swerdlow, 1993) failed to ameliorate the deficits to PPI produced by quinolinic acid lesions of the BLA

(Wan and Swerdlow, 1997). This would indicate that chemical lesioning of the basolateral amygdaloid complex most likely reduces PPI in rats by using neural substrates that are not dependent on DA transmission and it has been suggested that the shell of the nucleus accumbens may be involved in this process (Wan and Swerdlow, 1997).

Research indicates that PPI regulated at the shell of the nucleus accumbens is not DA-dependent but rather relies upon AMPA-mediated events since antagonism of AMPA receptors in the shell region has been shown to attenuate PPI (Wan, Kodsi, Caine and Swerdlow, 1995b). Thus, in addition to disrupting glutamatergic input to the ventral pallidum, the basolateral amygdaloid lesions or the blocking of NMDA receptors in the amygdala reported in the Wan and Swerdlow (1997) study most likely interfered with normal glutamatergic transmission to the shell of the accumbens. As a result, these experimental manipulations carried out on the amygdala could have produced changes in the shell of the nucleus accumbens that were similar to those caused by AMPA receptor blockade in the accumbens and this could have contributed to the disruption of PPI (Wan and Swerdlow, 1997).

An alternative explanation for the role of the nucleus accumbens regulating PPI put forward by Wan and Swerdlow (1997) involves a GABAergic neural mechanism that links the accumbens to the ventral pallidum (Swerdlow, Braff and Geyer, 1990). Support for this view comes from research demonstrating that the disruptive effects to PPI caused by intra-accumbens DA infusion can be reversed by the infusion of the $GABA_A$ agonist muscimol into the ventral pallidum which serves to activate GABAergic neurons and suppress the activity of DA in the nucleus accumbens (Swerdlow, Braff and Geyer, 1990). In any event, limbic dopaminergic abnormalities, increased amygdaloid excitability, and/or amygdaloid activation of the ventral pallidum or nucleus accumbens via a glutamatergic/dopaminergic or DA/GABAergic synergistic mechanism could possibly contribute to the bizarre emotionality, fearfulness and anxiety-like behaviours exhibited by schizophrenic patients (Swerdlow, et al., 1990; Jedema and Moghaddam, 1994; Haber and Fudge, 1997; Wan, Geyer and Swerdlow, 1995; Wan and Swerdlow, 1997; Feenstra, et al., 1998; Fendt, et al., 2000b). Thus, either excitation of the amygdala or abnormalities (i.e. DA hyperactivity) associated with this limbic region and other medial prefrontal areas mentioned above most

likely play a major role in the materialization of schizophrenia and in the expression of fear and stress responses.

4.31: Electrical Stimulation of the Amygdala, Unconditioned Fear Effects and Amygdaloid-Mediated Stress Responses

Excitation of amygdaloid neurons has been shown to play an integral role in mediating fear expression and electrical stimulation of the central amygdaloid nucleus produces a complex pattern of behavioural and autonomic changes that closely resemble a central fear state (Applegate, Kapp, Underwood, and McNall, 1983; Applegate, Kapp, Underwood, and McNall, 1985; Gelsema, McKitrick and Calaresu, 1987; Iwata, Chida, and LeDoux, 1987; Kapp, Gallagher, Underwood McNall and Whitehorn, 1982; Morgenson, and Calaresu, 1973; Rosen and Davis, 1988a; 1988b; Zbrozyna, 1972). For example, electrical stimulation of the amygdala or its central nucleus, produces behavioural freezing or a cessation of ongoing behaviour (Applegate, Kapp, Underwood, and McNall, 1983; LeDoux, 1987; Iwata, Chida, and LeDoux, 1987), changes in cardiovascular activity (Hilton and Zbrozyna, 1963; Reis and Oliphant, 1964; Kapp, et al., 1982; Applegate, et al., 1983; Kapp, Pascoe, and Bixler, 1984; LeDoux, Iwata, Cicchette, and Reis, 1988; Pascoe, et al., 1989; Iwata, Chida, and LeDoux, 1987), increased respiration (Applegate et al., 1983), and enhanced acoustic startle responding (Rosen and Davis, 1988a; 1988b; 1990; Koch and Ebert, 1993). Furthermore, both electrical and chemical stimulation of the central nucleus of the amygdala in awake and freely mobile laboratory animals gives rise to vagally-mediated gastric ulcer formation (Henke, 1980; Ray, Henke, and Sullivan, 1988a; 1988b), and stress-like changes in both heart and respiration rates and in mean arterial blood pressure (Brown and Gray, 1988; Iwata, Chida, and LeDoux, 1987; Morgenson and Calaresu, 1973). More specifically, chemical stimulation of the amygdala with L-glutamate and thyrotropin-releasing hormone produces changes in heart rate, blood pressure (Gelsema, et al., 1987) and in plasma catecholamines (Brown and Gray, 1988) that is similar in many respects to the changes produced following acute shock exposure (see Roozendaal, et al., 1991a).

Lesions confined to the central amygdaloid nucleus prevent the formation of stress-induced gastric ulcers (Henke, 1980a; Coover, Murison, Konow and Jellestad, 1992) and

significantly curtail the bradycardia response normally elicited by inescapable foot shocks (Roozendaal, Koolhaas, and Bohus, 1990). Also, lesioning of the medial or central amygdaloid nucleus significantly reduces the severity of both shock-induced and stress induced gastric ulceration in rats in most instances (Henke, 1980a; 1980b; 1981). However, ibotenate lesions of the central amygdaloid nucleus reportedly did not reduce the severity of ulceration produced by water restraint-stress indicating that other amygdaloid nuclei may play a prominent role in this type of stress-induced gastric ulcer formation (Coover, et al., 1992).

4.4: Amygdala's Role in Regulating Hypothalamic-Pituitary-Adrenal-Axis Activity during Periods of Fear and Stress

As stated previously, lesions of the central nucleus of the amygdala significantly attenuate the plasma catecholamine and stress hormone levels (Roozendaal, et al., 1991a; Feldman, Conforti, Itzik, and Weidenfield, 1994), possibly by destroying neurons or fibres of passage in the central nucleus that innervate the paraventricular nucleus of the hypothalamus, a brain area known to be involved in mediating the pituitary-adreno-cortical stress hormone response (Gray, Carney, and Magnuson, 1989; Chrousos, and Gold, 1992). In fact, the role played by the amygdala in regulating the functioning of the hypothalamic pituitary adrenal axis during periods of anxiety, stress or fear has been well documented in the research literature (Allen and Allen, 1974; Feldman and Conforti, 1981; Gray, Carney and Magnuson, 1989; Gray, 1993; Feldman, et al., 1994; Herman and Cullinan, 1997; Davis, 2000). For example, Feldman and Conforti (1981) demonstrated that adrenocortical responses associated with olfactory or sciatic nerve stimulation can be attenuated by lesioning the amygdala. Similarly, auditory and visual-sensory induced adrenocortical responses mediated at the level of the hypothalamic-pituitary axis were reported to be successfully blocked by lesions of either the central or medial amygdaloid nucleus (Feldman, et al., 1994). Furthermore, amygdaloid stimulation had been shown to have a facilitating effect on hypothalamically elicited flight responses in cats (Stokman and Glusman, 1970). Also, as was reported above, the increase in gastric ulceration formation and corticosterone levels along with changes in blood pressure can all be induced by either chemical or electrical stimulation of the amygdala (Brown and Gray, 1988; Iwata, Chida,

and LeDoux, 1987; Mogenson and Calaresu, 1973; Henke, 1980; Ray, Henke, and Sullivan, 1988a; 1988b; Gelsema, et al., 1987) and anatomical studies have shown that the amygdala projects to several hypothalamic regions either directly or indirectly via the BNST or the preoptic area (Krettek and Price, 1978a; Weller and Smith, 1982; DeOlmos, Alheid, and Beltramino, 1985). Thus, the excitatory effects on the hypothalamus produced by amygdaloid stimulation are likely mediated through the BNST or the preoptic area since these two areas receive extensive amygdaloid innervation from the central nucleus and project to the paraventricular nucleus of the hypothalamus (Krettek and Price, 1978a; Weller and Smith, 1982; DeOlmos, Alheid, and Beltramino, 1985; Sun, Roberts and Cassell, 1991).

Confirmation of the amygdala's excitatory influence on hypothalamic activity has been extensively demonstrated (Allen and Allen, 1974; Dunn and Whitener, 1986; Feldman, Conforti and Siegal, 1982; Feldman, et al., 1994 Manson, 1959; Matheson, Branch, and Taylor, 1971; Redgate, and Fahringer, 1973; Herman and Cullinan, 1997). For example, electrical stimulation of the central nucleus of the amygdala in several different animal species has been shown to elevate plasma corticosterone levels, suggesting that the amygdala has a modulatory role in influencing hypothalamic-pituitary-adrenal axis activity (Mason, 1959; Dunn and Whitener, 1986; Feldman, Conforti, and Siegal, 1982; Matheson, Branch, and Taylor, 1971; Redgate, and Fahringer, 1973; Steekleiv, Skaug, and Kaada, 1961). These increases in plasma corticosterone induced by amygdala stimulation can be blocked by bilateral lesions of the medial preoptic area, stria terminalis, or the BNST (Feldman, Conforti, and Saphier, 1990).

In addition, experiments incorporating immunohistochemistry, in situ hybridization, and radioimmunoassay techniques have shown that glucocorticoid receptors are colocalized with neurons that express corticotropin-releasing hormone (CRH) (Honkaniemi,, Pelto-huikko, Rechardt, Isola, Lammi, Fuxe, Gustafsson, Wikstrom and Hokflt, 1992) and chronic systemic administration of corticosterone has been shown to significantly increase CRH mRNA expression in the central nucleus of the amygdala and the BNST while at the same time inhibiting the activity of the hypothalamic CRH system at the level of the paraventricular nucleus (Makino, Gold, and Schulkin, 1994a; Makino, Gold and Schulkin, 1994b; Swanson and Simmons, 1989; Watts and Sanchez-Watts, 1995). Moreover,

corticosterone applied directly to the central nucleus of the amygdala elevated anxiety behaviours in rats, caused a robust increase in the basal level of corticotropin-releasing factor (CRF) mRNA expressed by individual neurons in the central nucleus, and significantly inflated the total number of cells in the central amygdaloid nucleus that produced a detectable CRF hybridization signal (Shepard, Barron and Myers, 2000). In this connection, it is noteworthy that peripheral corticosterone injections dispensed to rats for five consecutive days significantly reduced the threshold dose of intracerebroventricular CRH that was needed to cause CRH-enhanced startle (Lee, Schulkin and Davis, 1994). There is some indication that these high corticosterone levels may have sensitised existing CRH amygdaloid neurons and BNST CRH receptors and/or increased the numbers of CRH receptors by facilitating mRNA biochemical cascades in the central nucleus of the amygdala and BNST (Makino, et al, 1994a; 1994b; Swanson and Simmons, 1989; Watts and Sanchez-Watts, 1995). This may possibly explain why less CRH was needed by Lee and colleagues (1994) to produce a robust CRH-startle effect.

Indeed, there is research supporting the existence of such underlying biochemical events that involve corticosterone and CRH interactions in mediating stress and unconditioned fear responses (Makino, et al., 1994a; 1994b; Swanson and Simmons, 1989; Watts and Sanchez-Watts, 1995; Shepard, et al., 2000). For example, footshock and intracerebroventricular CRH administration have been shown to elevate *c-fos* mRNA levels in the amygdala, basal limbic forebrain regions and portions of the BNST (Campeau, Hayward, Hope, Rosen and Davis, 1991; Arnold, De Lucus, Shiers, Hancock Evans and Herbert, 1992; Anddreae, and Herbert, 1993; Rosen, Fanselow, Young, Sitcoske, and Maren, 1998). Similarly, intrusive forms of fear-inducing stressors such as mild restraint and exposure to footshock (that contain physiological and psychological elements) have been shown to activate both the hypothalamic and amygdala based CRH systems (Kalin and Takahashi, 1994; Pich, Lorand, Yeganeh, de Fonseca, Raber, Koob and Weiss, 1995; Hsu, Chen, Takahashi, and Kalin, 1998; Whitnall, 1993; Watts, 1996). As such, it appears that the amygdala and hypothalamus form a system that governs neuroendocrine balance and stress-induced changes.

However, some of the research in this field strongly suggests that psychological stress and anxiety enhance CRH mRNA expression and levels in the central nucleus of the

amygdala and the dorsolateral subdivision of the BNST, whilst more pure physiologically-based stressors (e.g. severe immobilization stress) increase paraventricular nucleus-based CRH mRNA levels without having any impact on CRH mRNA levels in the central nucleus of the amygdala (Pacak, Palkovits, Makino, Kopin and Goldstein, 1996; Makino, et al., 1999; also see Herman and Cullinan, 1997). Because psychological stress and not physiological stress actively enhances central amygdaloid and BNST CRH mRNA systems and increases CRH levels (Makino et al., 1999) it is highly probable that CRH released from the central nucleus will bind to the numerous CRH-1 receptor sites located in the basolateral nucleus and BNST and serve to augment or even exaggerate fear, stress responses and anxiety-like behaviours (De Souza, Insel, Perrin Rivier, Vale, and Kuhar, 1985; Wynn, Hauger, Holmes, Millan, Catt and Aguilera, 1984; Makino, et al., 1999). So even if there is some dissociation between psychological and physiological stressors it is still possible to envisage how the amygdala or BNST may be required to add a psychological component to the physiological stress responses mediated by the hypothalamus and enhance conditioned and unconditioned stress responses.

In fact recent neurophysiological research demonstrates that chronically stressed rats exhibit synaptic and morphological modifications in both the pyramidal and stellate neurons of the BLA and in the cells of the BNST (Vyas, Mitr, and Chattarji, 2001; Vyas, Mitra, Shankaranarayana Rao, and Chattarji, 2002; Vyas, Bernal and Chattarji, 2003). This neurosynaptic remodelling is characterized by an enhancement in the length of dendritic arborizations and a pronounced dendritic hypertrophy that is evident in BNST cells and in pyramidal and stellate neurons of the BLA (Vyas, et al., 2001; Vyas, et al., 2002; Vyas, et al., 2003). These types of neurochemical cascades and morphological changes induced by stress or fear-evoking stimuli at the level of the BNST and amygdala may provide some insight into how normal fear behaviours and stress responses could develop into long-term pathological anxiety (see Rosen and Schulkin, 1998; also see Davis, 1992c; Davis, Campeau, Kim and Falls, 1995; Lee and Davis, 1997; Davis and Lee, 1998; Davis, 2000; Walker, Toufexis and Davis, 2003).

Thus, on the whole, the above research findings lend support to the notion that the BNST, commonly referred to as the extended amygdala (see Alheid, et al., 1995) and certain amygdaloid nuclei may together constitute part of the neurocircuitry that is

responsible for mediating many unconditioned fear and anxiety-like behaviours and responses (see Davis, 1998; 2000; Davis, et al., 1995; Lee, Walker and Davis, 1997). This supposition is quite attractive for several reasons. First, the central nucleus of the amygdala sends numerous projections to the BNST (Krettek and Price, 1978a; Sun, Roberts and Cassell, 1991; Alheid, et al., 1995) and both regions target hypothalamic and brain stem regions that are known to produce many of the autonomic, behavioural and hormonal changes often associated with fear, anxiety and stress (Alheid, et al., 1995; Davis, 1998; 2000; Pitkänen, 2000; Feldman, et al., 1990; Mason, 1959; Knigge, 1961; Allen and Allen, 1974; Herman and Cullinan, 1997; Gray, 1993; Krettek and Price, 1978a; Weller and Smith, 1982; DeOlmos, Alheid, and Beltramino, 1985). More specifically, the BNST and central nucleus of the amygdala share strong reciprocal connections with the hypothalamus, ventral tegmentum, VTA, pontine parabrachial nucleus, and various nuclear regions of the vagus and nucleus of the solitary tract that are located at the level of the medulla (Hopkins and Holstege, 1978; Schwaber, Kapp, Higgins, and Rapp, 1982; Higgins and Schwaber, 1988; Holstege, Meiners and Tan, 1985; Moga and Gray, 1985; Moga, Saper, and Gray, 1989; Moga, Herbert, Hurley, Yasui, Gray and Saper, 1990; Veening, Swanson and Sawchenko, 1984; Gray and Magnuson, 1987; Wallace, Magnuson and Gray, 1992).

Research by Freedman and Cassell (1994) indicates that DA fibres are heavily distributed in the lateral central amygdaloid nucleus and in the dorsolateral subdivision of the BNST. Also, the BNST and central amygdaloid nucleus receive robust dopaminergic input from the VTA and ventral tegmentum and in turn send extensive projections back to these midbrain regions. For this reason it is highly probable that the BNST and CeA are involved in gating the level of emotional arousal during periods of fear and stress and in controlling the turnover of DA metabolites that occur in various mesolimbic and mesocorticolimbic regions following exposure to footshock, mild stressors or conditioned fear cues (Bjorklund and Lindvall, 1984; Hokfelt, Skirboll Rehfeld, Goldstein, Markey and Dann, 1980; Moore and Bloom, 1978; Wallace, et al., 1992; Swanson, 1982; Loughlin and Fallon, 1983; Cassell and Gray, 1989; Asan, 1996; Freedman and Cassell, 1994; Fadda, et al., 1978; Herman, et al., 1982; Deutch, et al., 1985 Claustre, et al., 1986; Goldstein, et al., 1996; Inglis and Moghaddam, 1999; Morrow, et al., 2000a,b).

In a similar fashion, nociceptive input reaching the BNST and central amygdaloid nucleus via the parabrachial nucleus may provide the BNST and central nucleus of the amygdala with sensory information that makes it possible for these two limbic areas to instigate the type of increases in respiratory activity that commonly occurs during periods of fear and anxiety (Bernard and Besson, 1990; Bernard, et al., 1993). Hence, fear-evoked alterations in respiration may be mediated by efferent projections from the BNST and central nucleus of the amygdala since lateral portions of the BNST and central amygdaloid nucleus have been shown to innervate the parabrachial nucleus, a region that is known to modulate respiratory function (Moga, Herbert, Hurley, Yasui, Gray and Saper, 1990; also see Krettek and Price, 1978; Price and Amaral, 1978; Hopkins and Holstege, 1978; Takeuchi, McLean and Hopkins, 1982).

Second, electrical or chemical stimulation of the BNST or central nucleus of the amygdala has been shown to cause significant increases in the levels of corticosterone in blood plasma samples (Matheson, et al., 1971; Brown and Gray, 1988; Dunn and Whitener, 1986; Dunn, 1987). Also, the involvement of both the BNST and central nucleus of the amygdala in autonomic and somatic regulation and in hypothalamic mediated neuroendocrine responses as they relate to fear, anxiety and stress has been thoroughly investigated and well established in the research literature (Knigge, 1961; Kawakami, Seto and Yoshida, 1968; Bonvallet and Bobo, 1972; Dunn, 1987; Galeno, 1983; Gray, 1989; Henke, 1979; 1988; Hilton and Zbrozyna, 1963; Iwata, Chida and LeDoux, 1987; Kapp, Frysinger, Gallagher and Haselton, 1979; Kapp, et al., 1982; LeDoux, et al., 1988; Smith and DeVito, 1984; Liang, Melia, Campeau, Falls, Miserendino, and Davis, 1992; Lee and Davis, 1995; 1997; Davis and Lee, 1998; Gerwitz, McNish and Davis, 1998). In this regard, it is noteworthy that stimulation of the medial hypothalamus produces robust *c-fos* immunoreactive labelling in the amygdala and BNST (Sandner, Oberling, Silveira, Di Scala, Rocha, Bagri and Depoortere, 1993) and generally leads to autonomic and behavioural effects that bear a striking resemblance to those caused either by central fear or stimulation of the amygdala (Sandner, Schmitt and Karli, 1979; Schmitt and Karli, 1980; Schmitt, Sandner and Karli, 1981; Matheson, Branch and Taylor, 1971; Iwata, et al., 1987; Kapp, et al., 1982; LeDoux, et al., 1988; for a review also see Davis, 1992a,b,c; Davis,

2000; LeDoux, 1992; 1993; 2000). These findings suggest that BNST- hypothalamic and amygdalo-hypothalamic neural circuitry is essential for mediating fear expression.

Third, anatomical and immunohistochemical studies have shown that the BNST and the central amygdaloid nucleus both have similar neurochemical properties and cellular architecture. This is evident by the fact that both limbic regions contain medium-sized spiny neurons (Alheid, et al., 1995; Cassell and Gray, 1989; Cassell, Gray and Kiss, 1986; Ju, Swanson and Simerly, 1989; Ju and Swanson, 1989; McDonald, 1982; McDonald, 1983; Shi, Modaressi and Cassell, 1990) that express reactivity to markers for a variety of neuropeptides such as vasopressin, substance P, somatostatin and calcitonin gene-related peptide (CGRP) to mention a few (Woodhams, Roberts, Polak, and Crow, 1983; Cassell, et al., 1986; Gustafson, and Greengard, 1990; Moga and Gray, 1989; Gray and Magnuson, 1987; Moga, et al., 1990; Haring, Humpel, Skofitsch, Krobath, Javorsky and Saria, 1991). For example, medial portions of the central nucleus of the amygdala and posterolateral regions of the BNST contain substance P and somatostatin and project mainly to the dorsal motor nucleus of the vagus and the nucleus ambiguus where they could influence bradycardia, urination, defecation and ulcer formation during times of fear, stress or anxiety (Cassell, et al., 1986; Gray and Magnuson, 1987; Moga, et al., 1989; Woodhams, et al., 1983; Gray, 1989; Higgins and Schwaber, 1983; Henke, 1988; Danielsen, Magnuson and Gray, 1989).

In contrast, more dorsolateral aspects of the BNST and central amygdaloid nucleus express high immunoreactivity for vasopressin, CGRP and cholecystokinin (CCK) antibodies and it is worth mentioning that these limbic areas exhibit copious dopamine-β-hydroxylase and tyrosine hydroxylase labelled immunoreactive fibres that likely originate from DA projection neurons in the VTA and ventral tegmentum (Oades and Halliday, 1987; Swanson, 1982; Loughlin and Fallon, 1983; Bjorklund and Lindvall, 1984; Hokfelt, et al., 1980; Moore and Bloom, 1978; Larssen and Rehfeld, 1979; McDonald, 1985; Haring, et al., 1991; Inagaki, Kito, Kubota, Girgis, Hillyard and MacIntyre, 1986; Ingram et al., 1989; Pu, et al., 1994). As a matter of fact, large quantities of CCK_B receptors are located in several amygdaloid nuclei (McDonald, 1985; Ingram, Krause, Baldino, Skeen, and Lewis, 1989; Schiffmann and Vanderhaeghen, 1991; Pu, Zhuang, Lu, Wu, and Hans, 1994) and the CCK agonist pentagastrin was shown to cause a dose-dependent increase in

acoustic startle amplitudes after it was infused into the amygdala of rats (Frankland, Josselyn, Bradwejn, Vaccarino and Yeomans, 1997). This finding suggests that CCK_B receptors in the amygdala play a role in mediating unconditioned fear responses and anxiety-like behaviours.

In a similar fashion, intra-amygdalar infusions of CGRP have been shown to cause significant changes in heart rate, blood pressure and plasma catecholamine levels that normally occur during a central fear-state (Brown and Gray, 1988). In addition, intracerebroventricular application or intra-amygdalar infusion of this neuropeptide (i.e. CGRP) typically results in fear-like defensive freezing behaviour surfacing even before any aversive stimuli (i.e. shock) is administered to experimental subjects (Poore and Helmstetter, 1996; Kocorowski and Helmstetter, 2001). Furthermore, infusion of the CGRP antagonist $hCGPR_{8-37}$ into the amygdala prior to final testing has been shown to block the expression of fear-induced freezing behaviour to an auditory CS but not to a contextual CS (Kocorowski and Helmstetter, 2001). These findings add credence to the view that biochemical events taking place in the BNST and the amygdala play a key role in the production of fear and anxiety-like behaviours and/or autonomic responses that are triggered by aversive events.

Fourth, repeated electrical stimulation of the VTA or lesions of the BNST have been shown to facilitate kindling and seizure activity in the amygdala (Gelowitz and Kokkinidis, 1999; Le Gal, Le Salle, Calvino and Ben-Ari, 1977; Stevens and Livermore, 1978) and electrical stimulation of the amygdala or epileptic discharge activity confined to this region often produces overwhelming feelings of fear in humans and generates fear-like responses in laboratory animals (Chapman, 1954; 1960; Halgren, Walter, Cherlow, and Crandall, 1978; Gloor, 1992; Trimble, 1991). Moreover, high frequency stimulation of VTA neurons which reportedly enhances kindling in the amygdala has also been shown to generate intense fear reactions in laboratory animals (Stevens and Livermore, 1978; Gelowitz and Kokkinidis, 1999). The enhancement of kindling produced by VTA stimulation likely involves a sensitisation process whereby VTA electrical stimulation excites amygdaloid neurons and lowers the current threshold required to induce kindling (Gelowitz and Kokkinidis, 1999).

This sensitisation process is probably mediated via the mesoamygdaloid DA pathway since electric stimulation of the VTA or exposure to fearful stimuli and anxiety provoking stressors have been shown to activate VTA DA neurons (Guarraci and Kapp, 1999) and increase the metabolism of DA in the amygdala and prefrontal cortex (Coco, et al., 1992; Inglis and Moghaddam, 1999; Goldstein, et al., 1996). The facilitating effects on amygdaloid kindling caused by BNST lesions can possibly be explained by the overall reduction in GABAergic-mediated inhibition that takes place once many GABAergic neurons confined to the BNST (Sun and Cassell, 1993) have been destroyed by lesioning. Thus, it is plausible that the destruction of GABAergic neurons intrinsic to the BNST may produce an overall lowering of excitability thresholds or somehow alter the way information is processed and stored within the BNST-amygdala fear and anxiety circuit.

This view is quite appealing since high concentrations of GABA are found in the reciprocal pathways between the BNST and amygdala (Gray, 1989; Sun and Cassell, 1993) and since BNST lesions have been shown to diminish most of the amnesic side effects that amygdala stimulation produces on avoidance responses when stimulation is administered shortly after fear training has been terminated (Liang and McGaugh, 1983). In addition, infusions of the $GABA_A$ antagonist bicuculline methiodide into the BLA, a region that is well connected to the BNST, produced long-term increases in heart rate and blood pressure that lasted for several weeks after drug infusions were made (see Sanders and Shekhar, 1991; 1995a). This indicates that antagonism of inhibitory GABAergic systems leads to increased excitation that is similar in nature to that produced by long-term stress. Thus, the BNST-amygdala fear and anxiety circuit seems to exert a powerful influence over autonomic, behavioural, and neuroendocrine responses that are associated with stress, fear, and anxiety. The next section will examine how CRH-mediated events in the amygdala and BNST are linked to fear and anxiety. Particular emphasis will be placed on how BNST and amygdala interactions may be involved in producing anxiety-like CRH and light-enhanced startle effects.

4.5: "The Amygdala and the Extended Amygdala": The Role of the Amygdala and BNST in Mediating Unconditioned Fear and Anxiety Behaviours

During stressful events or periods of intense anxiety CRH is released into a number of nuclei within the limbic system and serves to produce a constellation of behaviours that are indicative of fear and anxiety (Sutton, Koob, Le Moal, Rivier, and Vale; 1982; Menzaghi, Heinrichs, Pich, Weiss and Koob, 1993; Wiersma, Baauw, Bohus, et al., 1995; Makino, Shibasaki, Yanauchi, Nishioka, Mimoto, Wakabayashi, Gold and Hashimoto, 1999; Shepard, Barron, and Myers, 2000). The central nucleus of the amygdala has been shown to contain numerous CRH producing neurons that project to receptors located in the BNST (Swanson, Sawchenko, Rivier and Vale, 1983; Sakanaka, Shibasaki, and Lederis, 1986; Gray, 1993) and activation of the BNST by the amygdala could possibly be involved in the expression of unconditioned fear and anxiety. Research from several different laboratories has consistently shown that intracerebroventricular (ICV) infusions of corticotrophin-releasing hormone (CRH) cause physiological arousal and behavioural responses that are comparable in many respects to those generally observed when animals are exposed to intense stress or fearful stimuli (Swerdlow, Geyer, Vale, and Koob, 1986; Dunn and Berridge, 1990; Koob and Bloom, 1985; Tazi, Dantzer, Le Moal, Rivier, Vale, and Koob, 1987; Liang, Melia, Miserendino, Falls, Campeau and Davis, 1992a; Wiersma Bohus and Koolhaas, 1993). For example, CRH significantly increases the level of aggression and fighting amongst rats stressed by aversive shock (Tazi, et al., 1987) and serves to augment stressful behavioural responding to novel environments (Britton, Koob, Rivier and Vale, 1982). Also, laboratory animals that received direct CRH infusions into the central nucleus of the amygdala exhibited dramatic increases in the rate of cardiac activity when compared to control animals treated with the vehicle solution (Wiersma, et al., 1993). In a similar fashion, CRH microinfusions into the central nucleus of the amygdala in rats confined to a home cage environment produced increased arousal, excessive grooming, and behavioural activation that are a characteristic feature of anxiety (Wiersma, Baauw, Bohus, et al., 1995). Moreover, microinfusion of corticotropin-releasing factor (CRF) into the CeA has been reported to cause profound autonomic, hormonal and behavioural changes that are very similar in nature to the kind of effects induced by intense fear or anxiety (Gray, 1989).

Conversely, direct microinfusion of CRH antagonists (e.g. alpha-helical CRH 9-41) into the amygdala generally reduces emotionality, anxiety and stress-related responding in socially defeated rats, rats suffering from alcohol withdrawal or rats that have been exposed to stressful environments (Heinrich, Pich, Miczek, et al., 1992; Rassnick, Heinrichs, Britton, and Koob., 1993; Landgraf, Gerstberger, et al., 1995; Swiergiel, Takahashi, and Kalin, 1993). Furthermore, infusion of CRH antagonists into the central nucleus of the amygdala significantly reduces the degree of stress-evoked freezing exhibited by rats (Sweirgiel Takahashi, and Kalin, 1993). Most importantly, intracerebroventricular infusion of CRH has been shown to significantly increase acoustic startle responding above and beyond normal baseline startle levels and this anxiogenic behavioural effect can be reversed by the intraventricular infusions of alpha-helical CRH (9-41) a competitive CRH antagonist, pretreatement with chlordiazepoxide (Swerdlow, et al., 1986; Swerdlow, Britton and Koob, 1989) or by electrolytic lesioning of the central nucleus of the amygdala (Liang, Melia, Campeau, Falls, Miserendino and Davis, 1992b).

Since it has been well established that CRH binding sites are located on amygdaloid neurons and amygdaloid neurons may release CRH (Swanson, et al., 1983; De Souza, Insel, Perrin, Rivier, Vale and Kuhar, 1985; Sakanaka, Shibasaski and Lederis, 1986; Uryu, Okumura, Shibasaki, 1992) it is not surprising that bilateral electrolytic lesioning of the amygdala has been shown to significantly reduce the excitatory effects of CRH on startle (Liang, Miserendino, Melia and Davis, 1989; Liang et al., 1992b). Based on the results of the Liang et al., (1989; 1992b) studies and the work of various others who have studied the unconditioned effects of CRH (Wiersma, et al., 1993; Swerdlow, et al., 1986; 1989), it initially appeared as though the central nucleus of the amygdala was the crucial brain region responsible for mediating CRH-elevated startle.

One early plausible explanation offered for the marked attenuation of CRH effects on startle following amygdala lesioning was that the amygdala shares a close anatomical relationship with the parabrachial nucleus, a region that has been shown to enhance acoustic startle responding after receiving direct infusions of CRH (Liang, et al., 1989; Davis, 1992a,b; Davis et al., 1995). This early model proposed that the projections of the parabrachial nucleus to the central nucleus of the amygdala formed a complex neural circuit. Within this circuit, excitation of the parabrachial nucleus by CRH was thought to

activate the amygdala which in turn triggers further autonomic and neurochemical changes that elevate startle and produce the behavioural correlates that are typically associated with fear and anxiety (see Davis, 1992a,b,c). In essence, the blockade of the CRH-enhanced startle that followed lesions of the central nucleus of the amygdala was believed to be caused by interruption of the parabrachial-amygdalar neurocircuitry at the level of the amygdala (Davis, 1992a,b,c). At the outset this notion seemed quite plausible, given the fact that research demonstrated evidence of corticotropin-releasing neurons in the central nucleus of the amygdala projecting to the parabrachial nucleus (Moga and Gray, 1985; Sakanaka, et al., 1986). Thus, it initially appeared as though the central nucleus of the amygdala was the primary receptor area where CRH acted to potentiate acoustic startle responses and produce various other anxiety-like behaviours.

However, additional research indicated that there were potential difficulties with the view that the central amygdaloid nucleus neurons solely mediate CRH-enhanced startle. First, excitotoxic NMDA lesions carried out on either the ventral hippocampus or the BNST were shown to block CRH-enhanced acoustic startle responding (Lee and Davis, 1995; also see Davis, Walker and Lee, 1997) indicating that a neural circuit that included the ventral hippocampus, fornix, BNST and RPC likely was involved in mediating the CRH-induced elevation of startle given the high degree of anatomical connectivity between these brain regions (Canteras and Swanson, 1992; Cullinan, Herman and Watson, 1993; Amaral and Witter, 1995; Davis, Walker and Lee, 1997). Second, local infusion of CRH into either the central nucleus of the amygdala or ventral hippocampus does not significantly enhance acoustic startle amplitudes above baseline levels (Liang, et al., 1992; Davis, Walker and Lee, 1997). However, similar infusions made into the BNST did cause a marked increase in startle amplitudes that closely resembled the sustained excitatory effects on startle that are commonly produced following intracerebroventricular CRH application (Liang, et al., 1992; Davis, Walker and Lee, 1997). Third, fibre sparing neurotoxic lesions of either the central or basolateral amygdaloid nuclei were reported to have no impact on attenuating CRH-enhanced acoustic startle responding whereas, this unconditioned effect was blocked by excitotoxic NMDA lesions of the BNST (Lee and Davis, 1997). Fourth, direct infusion of a 6.0 μg dose of alpha-helical CRH (9-41) into the central nucleus of the amygdala did not block CRH-enhanced startle or FPS, however the

same concentration of this CRH antagonist microinjected into the BNST successfully blocked the CRH-enhanced startle produced by intracerebroventricular infusions (Davis, et al., 1997; Lee and Davis, 1997; Davis, 1997; Davis and Lee, 1998). Finally, lesions of the BNST did not block cue specific conditioned freezing behaviour or FPS (LeDoux, Iwata, Cicchetti and Reis, 1988; Hitchcock and Davis, 1991) however, lesions to this area did block the long-term sensitization of acoustic startle responding as well as contextually conditioned defensive freezing behaviour (Davis, et al., 1995; McNish, et al., 1997).

Taken together, the above results not only provide strong corroborating evidence that the BNST, and not the amygdala, is the key neural substrate that is involved in controlling the startle enhancing effects produced by intracerebroventricular administered CRH but also indicate that there is a clear dissociation between the neural circuits that deal with unconditioned fear and anxiety-like responses and those that deal with conditioned fear behaviours that rely on associative learning (see Davis, 1996; Davis, et al., 1997; Davis, 1997; Davis, 1998; Davis and Shi, 1999; Lee and Davis, 1997; Davis and Lee, 1998). Support for this notion comes from several studies that have examined the neurobiochemical and neuroanatomical processes underlying a phenomenon called light-enhanced startle (Walker and Davis, 1997a; 1997b). In this paradigm, acoustic startle responding is significantly enhanced after rats have been exposed to bright-light conditions and since this effect does not depend on prior conditioning and does not diminish in magnitude across test sessions, it is probable that light-enhanced startle represents an unconditioned anxiety-like effect rather than a conditioned fear effect that develops as a result of learning (see Walker and Davis, 1997a).

Generally speaking, this paradigm takes advantage of the fact that bright lighting conditions may be an anxiety-inducing stimulus for nocturnal species such as rats and mice. This makes sense as these organisms generally show significantly reduced open field activity in highly illuminated environments (McLearn, 1960; Candland and Nagy, 1969; Nagy and Glaser, 1970; Valle, 1970; Walsh and Cummins, 1976), avoid white illuminated test compartments (Crawly and Goodwin, 1980; Costall, Jones and Kelly, 1989) and have higher corticosterone plasma levels and make fewer social contacts with cage mates in brightly lit and unfamiliar environments (File and Hyde, 1978; File and Peet, 1980). Indeed, many pharmacological agents such as diazepam, midazolam, chlordiazepoxide and

buspirone have been shown to decrease several different behavioural and physiological measures of fear and anxiety including those caused by bright-light conditions when these compounds are administered either via the peripheral route or directly by infusion into the lateral and basolateral nuclei of the amygdala (Hodges, Green and Glenn 1987; Nagy, Zambo and Decsi, 1979; Petersen and Scheel-Kruger, 1982; Petersen, Scheel-Kruger and Petersen, 1982; Braestrup and Scheel-Kruger, 1985; Shibata, Yamashita and Yamamoto et, al., 1989; Thomas, Lewis and Iversen, 1985; Helmstetter, 1993; Costall, et al., 1989; Pesold and Triet, 1995; Green and Vale, 1992; Sanders and Shekhar, 1995).

In the case of light-enhanced startle, peripheral buspirone injection (5mg/kg) given subcutaneously was shown to completely suppress bright-light potentiated startle (Davis, et al., 1997b). Additionally, drugs that create anxiety in human subjects (e.g. yohimbine and piperoxane) have been shown to augment noise-alone acoustic startle responsiveness in laboratory rats (Davis, Redmond and Baraban, 1979). Moreover, the inverse benzodiazepine agonist β-carboline DMCM reportedly increased FPS in rats (Hijzen and Slangen, 1989), whereas benzodiazepine agonists such as diazepam and midazolam administered peripherally, blocked FPS (Davis, 1979; Berg and Davis, 1984; Hijzen and Slangen, 1989) and in some instances reduced baseline startle responding as well (Berg and Davis, 1984). In a related manner, either intraperitoneal or intra-basolateral amygdaloid administration of diazepam was shown to increase the response decrement in acoustic startle responding that was sensitised by extremely loud and aversive noise bursts (Young, Helmstetter, Rabchenuk, and Leaton, 1991).

Although some of the research discussed above has shown that the anxiolytic action of benzodiazepines may occur in the amygdala, it is important to highlight the fact that benzodiazepine compounds still have the capacity to produce anxiolytic effects even when the central nucleus of the amygdala and other neighbouring nuclei have been lesioned (Kopchia, Altman, Commissaris, 1992; Davis, 1994; Treit, Pesold, and Rotzinger, 1993; Yadin, Thomas and Strickland, 1991). While it is true that the anxiolytic effects of benzodiazepine drugs occur after these compounds are infused into the basolateral amygdaloid complex but not after infusion into the central nucleus (Petersen and Scheel-Kruger, 1982; Scheel-Kruger and Petersen, 1982; Petersen, Braestrup and Scheel-Kruger, 1985; Thomas, Lewis and Iversen, 1985; Green and Vale, 1992; Pesold and Treit, 1995), it

is somewhat disconcerting that benzodiazepines are still able to exert their anxiolytic effects when large portions of the amygdala have been damaged by lesioning or when these compounds are infused into the VTA (Gifkins, Greba and Kokkinidis, 2002). This situation is made more complicated by the fact that benzodiazepine infusion into the central nucleus of the amygdala does produce anxiolytic effects and does dampen fear responding in some circumstances (Shibata, Kataoka, Yamashita, et al., 1986; Shibata, Kataoka, Gomita, et al., 1982). However, this may depend on several factors such as the type of behaviour that is being assessed at the time of testing, the volume and dose of drug infused, the relative potency of the benzodiazepine being used and the point in time when testing is initiated after drug infusion.

With regards to the potency of benzodiazepines, infusion of the low potency benzodiazepine flurazepam (40.0 μg) into the amygdala failed to produce anxiolytic effects in a water lick suppression test (Shibata, Yamamoto, Ozaki, and Ueki, 1989), whereas infusion of more potent benzodiazepines such as midazolam (10.0 μg) or diazepam (1.0 μg) into the amygdala were shown to produce significant anxiolytic effects on this behavioural measure (Petersen, Braaestrup and Scheel-Kruger, 1985; Shibata, et al., 1989). Despite the fact that most research indicates that the anti-anxiety effects of benzodiazepines are mediated at the level of the amygdala which has been shown to contain numerous benzodiazepine receptors (Niehoff and Kuhar, 1983; Thomas, Lewis and Iversen, 1985), the nagging issue still remains that benzodiazepine drugs can produce anti-anxiety effects even when large portions of the amygdala have been damaged by lesioning.

Hence, the experimental results demonstrating the BNST and not the central nucleus of the amygdala is involved in mediating CRH-enhanced startle adds weight to the argument that brain regions other than the amygdala may be involved in mediating certain anxiety behaviours and unconditioned fear effects (see Liang, et al., 1992a b; Lee and Davis, 1995; Davis, et al., 1997; Davis, 1997; 1998; Wallace and Rosen, 2001; Fendt, Endres and Apfelbach, 2003). This has caused some in the scientific community to rethink or at least re-evaluate the notion that the amygdala is solely responsible for mediating unconditioned fear and anxiety-like behaviours (Davis, Walker and Lee, 1997; Davis, 1997; Davis, 1998; Wallace and Rosen, 2001; Fendt, et al., 2003). As a result, attention turned to the BNST and certain key amygdaloid nuclei as the likely candidates.

Recently some scientists have suggested that anxiety-like behaviours such as light-enhanced startle and unconditioned freezing to predator odours may be mediated by the BNST and certain specialized regions of the amygdala (see Walker and Davis, 1996; Walker and Davis, 1997a, b; Davis, 1997; Davis, Walker and Lee, 1997; Davis, 1997; 1998; Davis and Lee, 1998; Davis and Shi, 1999; Wallace and Rosen, 2000; 2001) and there are several research findings that tend to support this view. For example, infusion of the AMPA/kainate antagonist NBQX into either the basolateral nucleus of the amygdala or the BNST caused a significant reduction in the levels of light-enhanced startle responding without producing any dampening of startle responsiveness whatsoever (Walker and Davis, 1997b). In stark contrast, similar infusions of NBQX into the central amygdaloid nucleus failed to produce any significant suppression of light-enhanced startle (Walker and Davis, 1997b). Also, infusion of NBQX into the BNST has been shown to be ineffective in blocking FPS, whereas infusion into the central nucleus of the amygdala has been shown to consistently attenuate the expression of FPS (Kim et al., 1993; Davis, Walker and Lee, 1997).

With regards to the unconditioned or innate defensive freezing response to the predator odour trimethylthiazoline (TMT, a chemical compound derived from fox faeces), research has shown that either neurotoxic lesions or temporary inactivation of the amygdala with muscimol was ineffective in blocking TMT-induced freezing behaviour (Wallace and Rosen, 2001; 2003; Fendt, Endres and Apfelback, 2002). In stark contrast, chemical inactivation of the BNST with muscimol completely attenuated the TMT-induced defensive freezing response (Wallace and Rosen, 2003). This finding is generally consistent with the work of Walker and Davis, (1997b) who demonstrated that light-enhanced startle could be blocked by the inactivation of the BNST with infusions of the glutamatergic receptor antagonist NBQX. The only discrepancy was that the unconditioned or anxiogenic effect on startle caused by bright light exposure could also be attenuated by NBQX infusions into the BLA, whereas, neural inactivation of the central and lateral amygdaloid nuclei with muscimol failed to block TMT-induced freezing behaviour (Wallace and Rosen, 2003). Thus it appears that different nuclei and subnuclei of the amygdala are involved in mediating different kinds of unconditioned/innate fear responses and anxiety-like behaviours.

Any inconsistent results between the defensive freezing and potentiated startle paradigms can, according to Wallace and Rosen (2003), be influenced by the type of sensory modality (i.e. predator odour vs. bright light illumination vs. white-noise) that is used during testing as different behavioural eliciting stimuli recruit and tap into distinct sensory systems located within the assorted nuclei of the amygdala (Uwano, et al., 1995; Ono, et al., 1995). Despite the minor divergence between the freezing and startle paradigms as it relates to defining the role played by the basolateral nucleus in mediating unlearned fear responses, it is necessary to understand that for the most part the experimental results obtained from these two paradigms are congruent when the involvement of the BNST in unconditioned fear and anxiety responding is taken into consideration. For example, temporary inactivation of the BNST blocks both light-enhanced startle and TMT-induced unconditioned defensive freezing (Walker and Davis, 1997b; Fendt, et al., 2002; 2003). In addition, neurotoxic lesions of the BNST completely block CRH-enhanced startle, whereas, similar lesions to either the central nucleus or the basolateral amygdaloid complex (i.e. lateral and basal nuclei) were without effect (Lee and Davis, 1997). The experimental finding of Lee and Davis (1997) closely parallels the combined results obtained by the Wallace and Rosen (2001) and the Fendt, et al., (2003) studies in the sense that TMT-induced unconditioned freezing, was left intact after neurotoxic lesioning or temporary chemical inactivation of the lateral and central amygdaloid nuclei (also see Fendt, et al., 2002). Thus, it seems as though lesioning or chemical inactivation of the BNST blocks unconditioned fear responses and anxiety-like behaviours in both the potentiated startle and defensive freezing paradigms.

At present, the preponderance of scientific evidence seems to suggest that the BNST is highly involved in mediating unconditioned fear and anxiety-like behaviours and that there is some dissociation between the amygdala and BNST as far as conditioned and unconditioned fear/anxiety is concerned (Davis, 1998; Davis and Lee, 1998; Davis, Walker and Lee, 1997; Lee and Davis, 1997; Walker, et al., 2003). Despite this fact, the participation of the amygdala in unconditioned fear responding and anxiety should not be simply dismissed since both the BNST and amygdala are brain areas that have similar receptor subtypes, cellular composition and morphology and efferent projections that target hypothalamic and brain stem nuclei known to be involved in producing many of the

behavioural correlates and autonomic responses that are commonly associated with fear, anxiety, and stress (Hopkins, 1975; Hopkins and Holstege, 1978, Krettek and Price, 1978; Veening, et al., 1984; Van Der Kooy, et al., 1984; Moga and Gray, 1985; Rosen, et al., 1991; Alheid, et al., 1995; McDonald, 1998; Davis, 2000; Feldman, et al., 1990; Pitkänen, 2000; Lee and Davis, 1997; Herman and Cullinan, 1997; Makino, et al., 1999). It is also important to point out that excitation of amygdala neurons augments several unconditioned fear effects including acoustic startle responding (Rosen and Davis, 1988a; Boulis and Davis, 1989; Koch and Ebert, 1993; Koch, 1993) possibly by increasing the firing rate of neurons in the nucleus reticularis pontis caudalis that are known to be influenced by auditory stimuli (Koch and Ebert, 1993; Koch, 1993; Lee, López, Meloni, and Davis, 1996; Davis, et al., 1982a).

4.6: Electrical Stimulation of the Amygdala, the Sensitization of Startle, and Unconditioned Fear Responses

As has been previously mentioned, electrical excitation of amygdaloid neurons generally provokes fear-like responses in laboratory rats. In the startle paradigm, brief electrical stimulation of the amygdala has been shown to significantly enhance the amplitude of the acoustic startle response in rats using current intensity levels (0 -400 μA range) that did not produce observable motor responses in any of the animals when tested alone without a startle stimulus (Rosen, and Davis, 1988a). The stimulation duration of 25 ms used by Rosen and Davis (1988a) was also below that used to generate kindling in rats (Handforth, 1984) suggesting that the increase in startle could not be attributed to convulsions that occur during seizure activity. In addition, low to moderate levels of electrical stimulation (0-160 μA range) of the caudal ventral amygdalofugal pathway which contains amygdaloid efferents that have been shown to project to the brainstem startle circuit at the level of the reticularis pontis caudalis (Inagaki, Kawai, Matsuzaki, Shiosaka, and Tohyama, 1983; Davis, Gendelman, Tischler, and Gendleman, 1982; Rosen, Hitchcock, Sananes, Miserendino, and Davis, 1991; Lee, López, Meloni, and Davis, 1996) also caused similar increases in the acoustic startle response amplitude of rats (Rosen, and Davis, 1988a; Koch and Ebert, 1993). Moreover, electrical stimulation of the central nucleus of the amygdala was reported to cause increases in the firing rate of auditory

sensitive neurons in the nucleus reticularis pontis caudalis (Koch and Ebert, 1993; Lee, et al., 1996; Davis, et al., 1982a) highlighting the ability of the amygdala to influence unconditioned reflexive responses.

Electrophysiological research also reveals that the enhancement of startle produced by electrical stimulation of the central nucleus of the amygdala in rats is temporal in nature and closely resembles the decrease in startle latencies and the increase in startle amplitudes typically produced by exposing rats to a naturally occurring fear stimuli or to a conditioned stimulus previously paired with foot shock (Rosen and Davis, 1988b; Cassella, Harty, and Davis, 1986; Davis, 1986). Startle to a white-noise burst measured electromyographically from the neck muscles usually has a response latency of around 7-8 ms and exposure to a fear eliciting CS (i.e. light previously paired with foot shock) significantly increased the mean startle amplitude measured electromyographically and decreased the response latency to only 5 ms (Cassella, et al; Davis, 1986). Unconditioned fear effects that are similar to those produced by a CS can also be mimicked by electrically stimulating the amygdala near the time of testing (Rosen and Davis, 1988a; 1988b; Rosen and Davis, 1990). For example, electrical stimulation of the central amygdaloid nucleus produced the greatest increase in startle when the stimulation was applied simultaneously with the onset of the startle eliciting stimulus or within 1.25 to 2.5 ms before or after the onset of the startle stimulus (Rosen and Davis 1988b).

Furthermore, electromyographic (EMG) recordings from the neck muscles demonstrated that electrical stimulation of the amygdala increased the primary EMG acoustic startle response magnitude in a manner similar to the levels produced by a conditioned fear stimulus (Cassella, et al., 1986), indicating that the amygdala and its efferent projections are involved in mediating the behavioural expression of both conditioned fear and unconditioned fear evoked reflexive responses. Further support for the involvement of the amygdala and the VAF pathway in mediating unconditioned fear effects comes from experimental studies which demonstrate that electrically elicited startle from various points along the startle pathway can be enhanced either by direct stimulation of the amygdala (Rosen and Davis, 1990; Koch and Ebert, 1993) or by the presentation of a brief series of aversive foot shocks just prior to the initiation of an electrically elicited startle response (Boulis and Davis, 1989).

In this regard, it is interesting to note that brief exposure to aversive foot shocks produces a constellation of unconditioned fear responses and behaviours that are similar in many respects to those produced following electrical stimulation of the amygdala or exposure to a conditioned fear stimulus (Davis, 1989; 1992a, b; 1993; Rosen and Davis, 1988a; Boulis and Davis, 1989). For example, rats exposed to a series of rapid unsignalled foot-shocks show significantly increased acoustic startle responding when compared to their pre-shock baseline startle levels (Davis, 1989; Richardson and Elsayed, 1998; Willick and Kokkinidis, 1995; Gifkins, Greba and Kokkinidis, 2002; Greba, Gifkins and Kokkinidis, 2001). This shock-induced increase in acoustic startle amplitudes is referred to as the shock sensitisation of startle and this paradigm has been widely used to study the sensitizing effects of foot-shock (Davis, 1989a), the neuroanatomical and pharmacological substrates involved in fear expression (Hitchcock, Sananes and Davis, 1989; Willick and Kokkinidis, 1995; Gifkins, Greba and Kokkinidis, 2002), and the role of contextual cues in fear conditioning (Davis, 1989; Richardson and Elsayed, 1998; Greba, Gifkins and Kokkinidis, 2001; Greba, Gifkins and Kokkinidis 2001 unpublished observations).

Indeed there has been some debate in the research literature (see Davis, 1989; versus Richardson and Elsayed, 1998) on whether shock sensitisation is truly an unconditioned fear effect or whether it simply represents a form of rapid contextual conditioning to the startle apparatus where foot-shock is delivered before testing. As was mentioned earlier, unpublished research from our laboratory (Greba, Gifkins and Kokkinidis, 2001), suggests that the shock sensitisation of startle may contain elements of both conditioned and unconditioned fear. This seems plausible since rats that received 10, 30, or 60 unsignalled foot-shocks in a context that was very different to the startle testing apparatus were still able to exhibit significant increases in acoustic startle amplitude when tested in the startle chambers that had not been explicitly paired with the foot shock and when compared to their preshock startle levels or to control animals that received no unsignalled foot shocks. However, it is important to point out that rats exposed to unsignalled foot-shocks in the startle apparatus showed significantly higher levels of shock sensitisation than out of context shocked rats and this sensitisation effect was usually twice as large as the shock sensitisation effect observed in the out of context shocked rats, thus highlighting the importance of contextual cues in augmenting startle responding (Greba, Gifkins and

Kokkinidis, 2001; unpublished results). Nevertheless, the finding that out of context unsignalled shock presentation has the capacity to significantly augment startle amplitudes is still very important since it suggests that the shock can produce unconditioned fear effects and perhaps even a memory-trace about the aversive nature of the shock (see Willick and Kokkinidis, 1995).

Keeping this in mind, it is plausible that the excitatory effect produced by shock exposure can not only become associated with a neutral stimulus to support classical fear conditioning but it may also cause sufficient arousal and neural sensitisation to produce an independent memory-trace of the shock experience in and of itself. This notion of a memory-trace for the foot shock is particularly important for two reasons. Firstly, it may to some degree explain why fear-extinguished animals show robust fear reinstatement following re-exposure to the UCS (foot-shock), (see Westbrook, Iordanova, McNally, Richardson and Harris, 2002; Rescorla and Heth, 1975; Gewirtz, et al., 1997). Secondly, it may help explain why the symptoms associated with phobias and post-traumatic-stress-disorder (PTSD) return after a brief exposure to events that are stressful or similar in nature to the feared stimulus (Rescorla and Heth, 1972; Westbrook, et al., 2002; see Foa and Dancu, 1994 in Zinbarg and Mineka, 2001; Fyer, 1998 regarding phobias and PTSD).

It is important to note that unpublished experimental results from our laboratory demonstrate that electrolytic lesions of the central and basolateral nucleus block the out of context shock sensitisation effect (Greba, Gifkins, and Kokkinidis, 2001; unpublished). This result may indicate that lesions of the amygdala may disrupt short-term pain memories associated with footshock exposure and thus prevent rats from exhibiting elevated startle levels. This result is consistent with previous studies that have demonstrated that electrolytic lesions of the central nucleus of the amygdala or the caudal ventral amygdalofugal pathway which carries central nucleus efferents to the brain stem startle circuit at the level of the RPC completely block the shock sensitisation of startle (Hitchcock, et al., 1989). In contrast, lesions of the lateral nucleus of the amygdala or the rostral ventral amygdalofugal pathway which contains central amygdaloid efferent fibres that innervate forebrain areas do not block the shock sensitisation of startle (Hitchcock et al., 1989). In addition, neurotoxic NMDA lesions confined to the most anterior portion of the basolateral nucleus of the amygdala were also reported to block the shock sensitisation

of startle, suggesting that neurons located in the anterior BLA may be involved in processing nociceptive information about the aversive quality of the shock during fear conditioning (Sananes and Davis, 1992). The anterior BLA does receive innervation from the granular and dysgranular layers of the posterior insular cortex as well as somatosensory and nociceptive information from various thalamic nuclei (McDonald and Jackson, 1987; Turner and Herkenham, 1991; Mascagni, et al., 1993; McDonald, 1998; Shi and Davis, 1997; Shi and Cassell, 1997; Shi and Cassell, 1998; Shi and Davis, 1999) making it a region that is well suited for the processing of pain information and triggering behavioural manifestations of fear (Shi and Davis, 1999).

4.7: Amygdala and Septum Interactions in the Mediation of Unconditioned Fear Responses

Septal lesions, like unsignalled footshock, have also been shown to increase the magnitude of the acoustic startle response presumably by removing its inhibitory control on the activity of amygdaloid neurons (Melia, Sananes and Davis, 1991; Lee, Lin, and Yin, 1988; Melia). The medial and lateral septal nuclei innervate the central nucleus of the amygdala (Volz, Rehbein, Triepel, Kneupfer, Strumpf, and Stock, 1990; Dudley, Lee and Moss, 1990; Russchen, 1982) and also send projections to the lateral, paraventricular, and mammillary nuclei of the hypothalamus (Meibach and Seigel, 1977; Sawchenko and Swanson, 1983; Silverman, Hoffman, and Zimmerman, 1981). The hypothalamic nuclei in turn project to the central nucleus of the amygdala, thus giving the septum the ability to influence amygdaloid activity indirectly as well as directly. The medial nuclei of the amygdala send projections back to the lateral septal nucleus while the central nucleus innervates the lateral septum more indirectly via its projections to the lateral hypothalamus and the BNST which in turn project to the septum to complete the neural circuit (Krettek and Price, 1978b; Sakanaka and Magari, 1989; Staiger, Nurnberger, and Pattern, 1989). In addition, the ventral hippocampus sends projections via the frimba of the fornix that pass through the septum on route to the BNST, a structure that has been shown to be critically involved in mediating both CRH and light-enhanced startle (Amaral and Witter, 1995; Canteras and Swanson 1992; Cullinan, et al., 1993; Davis, Walker and Lee, 1997; also see Davis, Walker and Lee, 1997; Davis and Lee, 1998).

It has been suggested that the enhanced acoustic startle responding generated by septal lesions may represent an increase in the unconditioned fear or anxiety-state (Lee, Lin and Yin, 1988; Melia, Sananes, and Davis, 1991; Miller and Treft, 1979) that is closely related to CRH-enhanced startle and is possibly caused by the removal of inhibitory control that is normally imposed on the amygdala by the septum. For example, electrical stimulation of the septum has been shown to cause marked reductions in heart rate, pituitary-adrenal function, and blood pressure (Covian, Antunes-Rodrigues, and O'Flaherty, 1964; Holdstock, 1967; Malmo, 1961) each of which is typically elevated in an animal experiencing an internal state of fear. In stark contrast, electrical stimulation of the amygdala generally produces completely opposite effects to that observed during septal stimulation (for a review see; Davis, 1992a,b,c; Rosen and Davis, 1988a). More importantly, electrolytic lesions of the central nucleus of the amygdala completely reversed the excitatory effects on acoustic startle produced by septal ablations (Melia, Sananes and Davis, 1991). Taken together, these results indicate that the amygdala mediates the excitatory effects on startle produced by both conditioned and unconditioned fear (Hitchcock and Davis, 1986; Hitchcock, et al., 1989; Greba, Gifkins, and Kokkinidis, 2001; unpublished work) however, the hypothesis suggesting an inhibitory role for the septal area on amygdalar function has not been confirmed using the FPS paradigm.

Thus although, past research has demonstrated that amygdala lesions tend to reduce the enhancement of emotionality produced by septal ablations in several paradigms used to measure fear-motivated behaviours (Kling, 1958; Kling and Meyer, 1958; Kleiner, Meyer, and Meyer, 1967; Schwartzbaum and Gay, 1966), more recent work using the FPS paradigm has shown that septal lesions do not alter the magnitude of FPS or prevent diazepam and buspirone from blocking FPS (Melia and Davis, 1991). Furthermore, research has demonstrated that not all anxiolytic effects produced by septal lesions are blocked by amygdala lesions (Treit, Pesold and Rotzinger, 1993; Decker, Curzon, and Brioni, 1995). For example, septal lesions generally produce anxiolytic-like effects in the elevated plus maze and defensive shock probe burying tasks (Treit and Pesold, 1990; Menard, and Treit, 1996a; 1996b; Treit, Pesold and Rotzinger, 1993) and research has shown that amygdala lesions do not modify these effects (Treit et al., 1993). Moreover, although electrolytic lesions of the septum were shown to block CRH-enhanced startle

(Davis, Walker and Lee, 1997) fibre sparing chemical lesions of the medial septal nucleus failed to block this unconditioned effect (Davis, Walker and Lee, 1997), indicating that septal innervation of the BNST does not seem to influence the CRH-startle effect to a significant degree.

Thus it appears that more experimental work is required to elucidate the complex interactions between the septum, amygdala and BNST in mediating fear and anxiety-motivated behaviours. However, it is interesting to note that neurotoxic NMDA lesions of the ventral hippocampus or the BNST were reported to block CRH-enhanced startle indicating that neurons in the ventral hippocampus could be involved in mediating the CRH-induced potentiation of startle (Davis, Walker and Lee 1997). As stated above, the hippocampus, septum and BNST are linked via the frimbra of the fornix (Amaral and Witter, 1995; Canteras and Swanson 1992; Cullinan, et al., 1993) and according to Gray's theory of anxiety, the septum and hippocampus may form the septo-hippocampal neural complex that may be involved in producing and controlling anxiety-like behaviours (Gray, 1982; 1991).

Indeed, there is certainly some support for Gray's neuropsychological model of anxiety given the fact that numerous similarities have been reported to exist between the outcomes produced by septal and hippocampal formation lesions on fear and anxiety-motivated behaviour (for a review see Gray, 1982; Gray and McNaughton, 1983) and this makes it possible that the septo-hippocampal system may influence CRH-enhanced startle. The only difficulty with this interpretation is that direct infusions of CRH into the ventral hippocampus were reported to have no effect on elevating acoustic startle amplitudes, whereas similar infusions into the BNST caused a dose-dependent increase in acoustic startle responses (Davis, et al., 1997; Lee and Davis, 1997). Also, an earlier study by Lee and Davis (1997) did report that NMDA lesions of the ventral hippocampus were not very successful in blocking CRH-enhanced startle even when the NMDA lesions produced extensive cell tissue damage to most of the twelve animals in the ventral hippocampus lesion group. Moreover, fibre sparing chemical lesions of the medial septum which is part of the septo-hippocampal anxiety circuit failed to block CRH-enhanced startle, whilst NMDA lesions of the BNST successfully blocked this CRH-induced acoustic startle effect (see Davis, Walker and Lee, 1997).

These results should not be taken to mean that Gray's (1982; 1991) proposed septo-hippocampal anxiety circuit is not involved in fear and anxiety, rather it may indicate that the septo-hippocampal system and especially the septum may be involved in raising activity levels and generating specific defensive action-oriented behaviours that are designed to cope with anxiety producing stimuli (Gray and McNaughton, 1983; Decker, et al, 1995; Treit and Menard, 1997). For example, septal lesions have been shown to cause hyper-reactivity and irritability indicative of septal rage (King, 1958; Fried, 1973; Gray and McNaughton, 1982; Decker, 1995), increase activity levels in an open arm elevated plus maze, and significantly decrease the time spent burying a shock probe (Decker, et al., 1995; Menard and Treit, 1996a; 1996 b; Pesold and Treit, 1992; Treit and Pesold, 1990; Triet, Pesold and Rotzinger, 1993; Triet and Menard) suggesting that fear and anxiety are significantly reduced by lesions of this region. Thus, burying of the shock probe may be one of the many anxiety reducing behavioural repertoires that falls under the control of the septal region. Because chemically-induced lesions of the septum (Walker, Lee and Davis, 1997) and even the ventral hippocampus do not block CRH-enhanced acoustic startle responding (see Lee and Davis, 1997) it is probably logical to assume that these regions play a limited role in the acoustic startle responses that are enhanced by CRH or bright-light administration. Thus, it appears as though the BNST may play a critical role in producing CRH-enhanced and light-enhanced startle but, as previously discussed, the excitation of amygdaloid neurons also seems essential for eliciting fear and anxiety-like responses and this phenomena is not just confined to vertebrates that are lower on the phylogenic scale but appears to apply to humans as well (Davis, et al., 1995; Gloor, Oliver, and Quesney, 1981; Halgren, Walter Cherlow and Crandel, 1978; Gloor, 1992; Davis, 1992).

4.8: Excitation of the Human Amygdala and its Link to Fear, Anxiety and Fear-Provoking Memories

Electrical stimulation of the human amygdala is reported to evoke feelings of fear or euphoria (Chapman, Schroeder, Guyer, Brazier, Fager, Poppen, Solomon, and Yakolev, 1954; Gloor, et al., 1981); however the valence of the emotion expressed likely depends on the location stimulated (see Halgren, et al., 1978). Electrical excitation of the amygdala

generally produces overwhelming feelings of fear or anxiety as well as various autonomic/visceral nervous system reactions that are customarily indicative of a central fear state (Chapman, Schroeder and Guyer, et al., 1954; Halgren, et al., 1978; Gloor, Oliver and Quensney, 1981). Similarly, abnormal amygdaloidal electrical discharge patterns caused by temporal lobe epileptic seizures also produce heightened autonomic arousal and behavioural effects that closely resemble a central fear state (Halgren, et al., 1978). Typically, these autonomic and behavioural changes are marked by heart palpitations, epigastric upset, mydriasis, pallor, increased respiration and an increased propensity to startle to sudden loud noises (Gloor, 1992; Davis, 1992).

Cognitive disruptions and disorders of thought and mood generally precede or begin with the onset of amygdaloidal epileptic discharge and these abnormalities in amygdala activity generally produce fearful hallucinations, frightful memory flashbacks, paranoid ideations and heightened levels of anxiety (Gloor, 1990; 1992; Gloor, et al., 1991; Crandall, Walter and Dymond, 1971; Halgren et al., 1978). Although many of these behaviours and responses are unconditioned it is still possible that not all behaviours and reactions provoked by electrical stimulation of the amygdala can be classified as unconditional because the stimulation of this limbic structure may be re-exciting old fear memories that have been laid down for quite some time. Thus, it is quite possible that some of the behavioural and emotional pathology associated with electrical discharge produced by epileptic seizures arises out of real fear memories (Gloor, 1990; 1992; Halgren, et al., 1978). This belief is supported by experimental data and publicized works that highlight the fact that a kindling-induced escalation of fearfulness of novel environments in rats can be reinstated by additional seizure activity if this neophobic response becomes degraded with the passage of time (see Kalynchuk, Pinel, Barr, Kippin, and Treit, 1997 in Depaulis, Helfer, Deransart, and Marescaux, 1997). As will be seen later, this notion of reexciting old fear memories by amygdaloid stimulation will form the basis of some of the experimental work carried out in this thesis but for now it is sufficient to state that the amygdala and BNST play a critical role in generating anxiety-like behaviours, unconditioned fear reactions and stress responses. However, as the next chapter will clearly point out, perhaps the greatest contribution of the amygdala is its crucial involvement in the acquisition and expression of conditioned fear.

Chapter 5

Pavlovian Fear Conditioning and the Role of the Amygdala in Conditioned Fear Learning and Expression

In a typical Pavlovian fear conditioning paradigm, a neutral stimulus (i.e. light or tone) that produces no significant emotional arousal in and of itself is repeatedly and contiguously paired with an aversive stimulus (i.e. footshock) called an unconditioned stimulus (UCS). Several such pairings cause a strong association between the previously neutral stimulus, now called a conditioned stimulus (CS), and the UCS (footshock) to be formed. Thus, during testing, the presentation of the CS alone is capable of eliciting a constellation of behavioural and physiological responses that are indicative of a central fear state and these are similar in many respects to those generated when the UCS is presented alone (Davis, 1992a,b,c; Kapp, et al., 1992; LeDoux, 1992). This form of learning is pervasive within the animal kingdom and has been demonstrated in most vertebrate species, including cats (Brady, Schreiner, Geller and Kling, 1954; Horvath, 1963) rats (Davis, 1992a,b,c; LeDoux, 1992; 1993; 2000; Davis and Astrachan, 1978; Davis, Schlesinger, and Sorenson, 1989; Falls and Davis, 1994), mice (Falls, Carlson, Turner and Willott, 1997; Desmendt, Garcia, and Jaffard, 1998), rabbits (Kapp, Fysinger, Gallagher and Haselton, 1979; Applegate, et al., 1982; Kapp, Wilson, Pascoe, Supple, and Whalen, 1990; Guarraci and Kapp, 1999), dogs (Pavlov, 1927), monkeys (Weiskrantz, 1958; Downer, 1961), and even humans (Ross, 1961; Spence and Runquist, 1958; Cuthberg and Lang, 1985; Hamm, Stark, and Vaitl, 1990; Grillon, Ameli, Goddard, Woods, and Davis, 1991; Hamm, Greenwald Bradley and Lang, 1993; Grillon, Ameli, Woods, Merikangas, and Davis, 1994; Lipp, Sheridan, and Siddle, 1994; Grillon and Davis, 1997).

Pavlov (1927) and several others (Konorski, 1948; Brown, Kalish and Farber, 1951; Davis, 1992a,b,c; Davis, 1989b; Davis, 1997; Falls and Davis, 1994; Fendt and Faneslow, 1999; Kock, 1999; LeDoux, 1992; 2000) who have extensively used classical conditioning techniques to uncover the neurobiological and behavioural processes underlying learning and memory functions as they relate to fear, emotionality, and motivational sensory stimuli (Konorski, 1948; Hebb, 1949; Bouton and Bolles, 1980; Falls and Davis, 1994; LeDoux, 1993; 2000) all maintain that simple CS-UCS pairings simultaneously establish two distinct

yet closely related types of associations or memories. As far as Pavlovian fear conditioning is concerned, the first deals primarily with the cue-specific memory involving the discrete CS (i.e. light) that predicts the onset of an aversive event (i.e. shock). The second deals with the contextual cues associated with the fear conditioning apparatus independent of the CS (Fanselow, 1980; 1982; Fanselow and Bolles, 1982; Fanselow, 1984; 1986; Davis, 1992; 2000; LeDoux, 1993; 2000; Fendt and Fanselow, 1999).

During testing, presentation of the CS either in the context where fear conditioning took place or in a context distinct to the fear conditioning apparatus effectively produces behavioural and autonomic changes that are indicative of a central fear state (see Davis, 1992a,b,c; LeDoux, 1992; 1993; 2000; Borowski and Kokkinidis, 1996; Fendt and Fanselow, 1999). In a similar fashion, simply returning an experimental subject to an environment that has been previously paired with foot shocks also elicits fear responding thus, indicating that a memory of the aversive event has become associated with the contextual cues of the conditioning apparatus (see Fanselow, 1980; 1986; Richardson and Elsayed, 1998).

Although research has demonstrated that these two types of fear memories have to some degree been neuroanatomically dissociated (i.e. amygdala lesions block both contextual and CS-specific fear, whereas dorsal hippocampus lesion or lesions confined to the accessory basal amygdaloid nucleus only block contextual fear) it is important to note that they do represent two separate but related emotional memories that may influence one another depending on the situation (Phillips and LeDoux, 1992; Selden, Everitt, Jarrard, and Robbins, 1991). As mentioned earlier, another interesting possibility is that a memory trace for the aversive nature of the footshock (i.e. UCS) may also be established during classical fear conditioning which may facilitate fear reinstatement after extinction training. In this connection it is interesting to note that Rescorla (1974) demonstrated that low levels of conditioned fear displayed by rats in response to a CS could be substantially increased by simply administering unsignalled footshocks that were more severe than the ones used during fear conditioning (i.e. neutral stimulus + UCS parings). This result indicates that a memory trace about the quality and intensity of a footshock (i.e. UCS) is established during classical fear conditioning since the presentation of a more aversive UCS alone can intensify conditioned fear responding in the presence of a mildly feared CS (see Rescorla,

1974). Thus, memories that deal specifically with the intensity and nature of the UCS may be stored in either the amygdala or some other limbic region that is closely interconnected with the amygdala.

5.1: Conditioned Fear Memories are Enduring but can be inhibited through Extinction Training

It is important to stress that emotional memories established through classical fear conditioning are acquired rapidly and are very enduring even after repeated testing or when the testing occurs up to 30 days after conditioning (Davis, 1992a,b,c; LeDoux, Xagoraris, and Romanski, 1990; MacKintosh, 1983; Falls and Davis, 1994; Greba and Kokkinidis, 2000; Greba, Gifkins, and Kokkinidis, 2001; Walker and Davis, 2000; Davis, Walker, and Myers, 2003). In some instances fear memories that are formed out of Pavlovian fear conditioning can be stored for prolonged periods of time (i.e. two or three years in the rat) and thus span the entire life time of the adult lab rat (Gale, Anagnostaras, Godsil, et al., 1999; Davis, et al., 2003; Gale, Anagnostaras, Godsil, Mitchell, Nozawa, Sage, Wiltgen and Fanselow, 2004). Furthermore, although the behavioural expression of fear can eventually be extinguished by the repeated presentation of non-reinforced CS trials during extinction training, it is important to note that this does not mean that the fear memory laid down during Pavlovian fear conditioning is forgotten or no longer exists as re-exposure to the aversive UCS in the conditioning and/or behavioural testing context causes a rapid reinstatement of conditioned fear responding (Pavlov, 1927; Konorski, 1948; Boutin, 1979; Bouton and Bolles, 1979a; 1979b; 1980; Bouton, 1983; Rescorla and Heth; 1975; Rescorla and Cunningham, 1977; Gewirtz, et al., 1997; Westbrook, Iordanova, McNally, Richardson and Harris, 2002; Davis, Walker, and Myers, 2003).

Typically, the extinction of a conditioned fear behaviour or response will occur after repeated presentation of the CS over time and it is believed that this form of inhibitory learning serves to mask the association that had been established between the CS and UCS during Pavlovian fear conditioning (Pavlov, 1927; Konorski, 1948; Westbrook, et al., 2002). Some researchers suggest that CS processing possibly declines during extinction training when the CS trials are repeatedly presented alone and it is hypothesised that this may lead to the CS actually becoming a good predictor of non-reinforcement (Pearce and

Hall, 1980). Others (Wagner, 1978; 1981) suggest that extinction training (i.e. repeated CS trials) could cause animals to blend together or make associations between the CS and the other background contextual cues, thus weakening the excitatory impact that the CS will have on future behavioural responses. There is also the suggestion that repeated CS presentations during extinction training without any reinforcement leads to inattention whereby animals start to ignore the CS simply because it is no longer a reliable predictor of the UCS (Lubow, Weiner, and Schnur, 1981). Still other researchers have gone as far as suggesting that extinction leads to a complete "erasure" of the original CS-UCS association and as a result makes it impossible to perform a conditioned response (see Estes, 1955). The major difficulty with the "erasure hypothesis" is that several experiments over the years have consistently demonstrated that re-exposure to the UCS can result in a reinstatement of extinguished responding (Pavlov, 1927; Rescorla and Heth, 1975; Bouton and Bolles, 1979 Rescorla and Cunningham, 1977; Westbrook, et al., 2002; Falls and Davis, 1992 in Falls et al., 1992; Gewirtz, et al., 1997). This finding seems to indicate that memories and especially fear memories are quite indelible and generally survive extinction training. Regardless of all the learning mechanisms or stimulus factors that are thought to be involved in fear extinction, there seems to be a great deal of consensus that they likely entail a new form of learning that masks or holds in check the fear memories that were established during fear conditioning (Konorski, 1948; Bouton and King, 1983; 1986; Bouton and Bolles, 1979; 1985; Rescorla and Heth, 1975 also see Rescorla, 2001; Davis, Falls, and Gerwitz, 2000).

In any event it can be stated, that while both fear extinction and conditioned fear acquisition represent forms of learning and behavioural expression that fall at opposite ends of the spectrum, both seem to rely heavily on the functional integrity of the amygdaloid neurons and NMDA receptors located in the basolateral nucleus (Sananes and Davis, 1992; Campeau and Davis, 1995; Walker and Davis, 2000; Goosens, and Maren, 2003; Falls, Miserendino, and Davis, 1992; Walker and Davis, 2002; Lee and Kim, 1998; Walker, Ressler, Lu and Davis, 2002a). For example, NMDA receptor antagonists infused into the basolateral complex prevent the acquisition of FPS and its extinction (Miserendino, Sananes, Melia and Davis, 1990; Falls, et al., 1992). Similarly, conditioned defensive freezing to auditory and contextual cues previously paired with aversive footshocks is

blocked by pretraining intra-amygdalar infusions of NMDA receptor antagonists (Goosens and Maren, 2003; Lee and Kim, 1998). Also, rats failed to extinguish a previously acquired defensive freezing response following the blockade of amygdaloid NMDA receptors with AP5 (Lee and Kim, 1998). Additionally, intra-amygdalar microinfusions of D-cycloserine, a partial agonist at the strychnine-insensitive glycine recognition site on the NMDA receptor complex that acts to enhance NMDA receptor activity, has been shown to facilitate the extinction of FPS in rats (Walker, Ressler, Lu, and Davis, 2002a). This discovery by Walker and associates (2002a) indicates that NMDA receptor activation may be necessary to help support this type of inhibitory learning.

5.2: Pavlovian Conditioned Fear Described

In general, conditioned fear can be described as the constellation of autonomic, behavioural, hormonal, neurochemical and neurosynaptic changes that take place during classical conditioning (e.g. light + footshock training trials) or are triggered by the presentation of a CS previously paired with the aversive UCS (Davis, 1992a,b,c; 1997; 2000; Kapp et al., 1992; LeDoux, 1978; 1992; 1993; 2000; Koch, 1993; Fendt and Fanselow, 1999). Following classical fear conditioning, presentation of a CS (i.e. tone or light) has the capacity to augment the acoustic startle response and elicit a number of conditioned emotional responses such as defensive freezing, defecation, piloerection, stereotyped increases in arterial pressure and heart rate, and the release of adrenal hormones into the blood stream (Brown, Kalish and Farber, 1951; Davis, 1992a,b,c; LeDoux, 1992; Fendt and Fanselow, 1999; Maren, 1999; LeDoux, 2000; Davis, 2000). A wealth of experimental evidence suggests that the amygdala is critically involved in both the acquisition and expression of conditioned fear (for reviews see Davis, 1992a,b,c; Davis, 1997; 2000; LeDoux, 1992; 1993; 2000; 2002; Fendt and Fanselow, 1999; Koch, 1999; Fanselow and LeDoux, 1999; Maren, 1999; Maren, 2003) and in the initiation and regulation of fear motivated responses (Blanchard, D. C., and Blanchard, R. J., 1972; Coover, Ursin, and Levine, 1973; Lukaszewska, Korcczynski, Markowska and Kostarczyk, 1980; Slotnick, 1973; Spevack, Campbell, and Drake, 1975; Ursin, Jellested, and Cabrera, 1981; Iwata, LeDoux, Meely, Arneric and Reis, 1986; LeDoux, Cicchetti, Xagoraris, and Romanski, 1990; Kemble, Blanchard D.C. and Blanchard R. J., 1990).

Thus, it is not surprising that the lateral and basolateral amygdaloid nuclei receive a wealth of highly processed auditory, visual, somatosensory, and nociceptive information from various thalamic nuclei and cortical areas such as the anterior inferior temporal lobe and the perirhinal cortex (PRh) (for a review see; McDonald, 1998; Pitkänen, 2000; Shi and Davis, 1997; 1998; 1999; Shi and Cassell, 1998a; 1998b; 1998c; Ono, et al., 1995; Bernard and Besson, 1990; LeDoux, et al., 1990; 1991; Turner and Herkenham, 1991; LeDoux, 1992; Romanski and LeDoux, 1993; Davis, 1992; Campeau and Davis, 1995; Mascagni, McDonald and Coleman, 1993; Fendt and Fanselow, 1999; Shi and Davis, 2001). The lateral and basolateral nuclei innervate the central nucleus of the amygdala which in turn projects to a variety of hypothalamic and brainstem areas that are involved in mediating specific autonomic and behavioural responses that are indicative of a central fear state (Pitkänen, Savander and LeDoux, 1997; Pitkänen, et al., 1995; Swanson and Petrovich, 1998; Davis, Falls, Campeau, and Kim, 1992; Davis, 1992a; LeDoux, 1992).

Lesions or pharmacological manipulations restricted to the lateral, basolateral, or central nucleus of the amygdala have been shown to interfere with the acquisition and expression of autonomic, behavioural, and reflexive conditioned fear responses (Blanchard and Blanchard, 1972; Kapp, Frysinger, Gallagher, and Haselton, 1979; Gallegher, Kapp, Pascoe, and Rapp, 1981; Gentile, Jarrel, Teich, McCabe, and Schneiderman, 1986; Hitchcock and Davis, 1986; Iwata et al., 1986; Miserindino, Sananes, Melia, and Davis, 1992; Sananes and Davis, 1992; Kim and Davis, 1993a,b; Kim, Campeau, Falls and Davis, 1993 Campeau and Davis, 1995; Lee, Walker and Davis, 1996; Waddington-Lamont and Kokkinidis, 1998; Gurarci, Frodhardt and Kapp, 2000, 2001; Greba and Kokkinidis, 2000; Greba, Gifkins and Kokkinidis, 2001;Walker and Davis, 2000; Fendt, 2001). Electrolytic or ibotenic acid lesions of the central and BLA completely block the expression of conditioned defensive freezing behaviour and conditioned hypoalgesia (Helmstetter, 1992; Helmstetter and Bellgowan, 1993). Also, temporary cryogenic inactivation of the central amygdaloid nucleus blocks aversively conditioned blood pressure and respiratory responses (Zhang, Harper, and Ni, 1986).

On the other hand, electrical stimulation of the amygdala or exposure to a fear-eliciting CS has been shown to significantly alter respiration rates and cardiovascular functioning in animals (Anand and Dua, 1956; Bonvallet and Gary Bobo, 1972; Applegate et al., 1983;

Harper, Frysinger, Trelease and Marks, 1984; Hilton and Zbrozyna, 1963; Stock, Ruupprecht, Stumpf and Schlor, 1981; Zhang, et al., 1986; Kapp, Gallagher, Underwood, McNall, and Whitehorn, 1982). This is important since increased respiration is considered to be a prominent physiological manifestation of fear in humans, especially those who suffer from panic disorders (Davis, 1998). The fact that chemical or electrical excitation of central amygdaloid neurons causes autonomic and behavioural responses that are similar in many respects to behaviours and responses elicited by a conditioned fear stimulus is significant (Reis, and Oliphant, 1964; Kapp, et al., 1982; Galeno and Brody, 1983; Iwata, et al., 1987; Rosen and Davis, 1988a,b; also see above) as many of the unconditioned fear effects mentioned previously can be incorporated into the pool of scientific information that allows us to understand the neural substrates involved in conditioned fear learning since they provide an uncontaminated measurement of amygdaloid activity during behaviour.

5.3: Fear-Motivated Behaviours, Conditioned Fear and Amygdala Lesioning

In terms of fear-motivated behaviours, lesions of the central nucleus of the amygdala have been shown to disrupt behavioural immobility in rats exposed to acute footshock or complex stimuli that signals the onset of inescapable footshock (Iwata, et al., 1986; Roozendaal, Koolhaas, and Bohus, 1991; 1990). Furthermore, lesions of the corticomedial and basolateral amygdalar groups impaired flight and escape responses in rats exposed to threatening environmental stimuli. Lesioning of these amygdaloid regions also attenuated defensive vocalizations and put a stop to the jumping and biting-attack responses induced by vibrissae stimulation (Kemble, Blanchard D. C., and Blanchard, R. J., 1990). Similarly, lesions of the amygdala or its central or basolateral nuclei have also been reported to cause significant deficits in conditioned active avoidance responding in laboratory animals (Brady, Schreiner, Geller and Kling, 1954; Coover, et al., 1973; Eclancher and Karli, 1980; Goldstein, 1974; Hovath, 1963; Schutz and Izquierdo, 1979; Thatcher and Kimble, 1966; Ursin, 1965 Yeudall and Walley, 1977) suggesting that the amygdala is involved in the learning and expression of behavioural and motor responses that make it possible for an animal to escape from dangerous or fearful situations. For example, Liang and colleagues (1982) demonstrated that posttraining amygdala lesions made two days, but not ten days, after inhibitory avoidance training impair inhibitory avoidance-retention. Similarly, either

large electrolytic lesions of the amygdaloid complex or small lesions confined to discrete nuclei within the amygdala prior to or just after passive avoidance training was shown to cause significant impairments in passive avoidance behaviour in rats and cats (Coover, et al., 1973; Grossman, Grossman, and Walsh, 1975; Liang et al., 1982; Pellegrino, 1968; Russo, Kapp, Holmquist, and Musty, 1976; Slotnick 1973; Swartzwelder, 1981; Ursin, 1965). Also, ibotenic acid lesions of amygdaloid neurons in the basolateral and central nucleus impaired passive avoidance learning, increased open field exploration and activity levels, and caused a marked increase in plasma corticosterone levels (Jellestad and Blake, 1985).

Amygdaloid lesioning, reversible inactivation, or posttraining drug infusions generally cause significant retention impairments in conditioned avoidance responding. Although, it is important to point out that some researchers have argued that these memory-impairing effects can be ameliorated either by providing animals with extensive training prior to lesioning or drug infusions (Goldstein, 1974; Parent, Avila, and McGaugh, 1995a; Parent, Tomaz, and McGaugh, 1992; Parent, West, and McGaugh, 1994; Thatcher and Kimble, 1966; also see McGaugh, Introini-Collison, Cahill, Kim and Liang, 1992) or by inducing lesions of the amygdala at least ten days after the inhibitory avoidance training has taken place (Liang, et al., 1982; Liang, 1991; McGaugh, et al., 1992; Parent, et al., 1994). For instance, Parent, Quirate, Cahill and McGaugh, (1995b) and several others (Parent, Avila, and McGaugh, 1995a; Parent, West and McGaugh, 1994) have demonstrated that inhibitory avoidance retention is spared if posttraining amygdala lesions were made several days after training or if rats are exposed to an extensive inhibitory avoidance training regime before lesioning takes place.

These findings have led some researchers to speculate that the amygdala may only be temporarily involved in the fear-motivated learning and behavioural processes (see McGaugh, Parent, Cahill, 2000). In other words, these researchers take the position that the amygdala modulates fear memories stored elsewhere in brain areas such as the prefrontal cortex or insula cortex. According to this view, the amygdala is not necessarily the site where plasticity occurs and where fear memories are laid down, but rather a limbic structure that modulates and retrieves fear memories stored in other brain areas (see McGaugh, et al., 1992; McGaugh, et al., 2000). The amygdala, according to McGaugh and

colleagues and others (Parent, et al., 1992; 1994; 1995a,b; Packard and Teather, 1998; Cahill, 1999) is thought to modulate multiple memory systems in the brain. As a result, these research scientists believe that fear memories stored in other brain areas are likely transmitted to the amygdala and therefore may encourage the amygdala to initiate behavioural actions and biologically adaptive responses (i.e. hypervigilence, increased cardiovascular activity, avoidance and escape) when threatening fear cues are detected.

Although this view has caused some debate as to the exact nature of the amygdala's role in the long-term storage and retrieval of fear memories, it can be stated that the view expressed by McGaugh and his fellow scientists is not necessarily incompatible with some of the research findings obtained from the FPS paradigm. For example, Kim and Davis (1993a) showed that bilateral amygdaloid lesions blocked FPS in rats that received extensive training but did not prevent fear reacquisition if lesioned rats were given additional fear conditioning trials. These results indicate that fear memories stored in other brain areas may take over mediating fear expression at the level of the brainstem startle circuit even if the amygdala has been damaged (Kim and Davis, 1993a). However, these findings should not be taken to mean that the amygdala is unnecessary for fear learning and expression or that it does not undergo changes in synaptic efficacy, rather it should highlight the fact that the amygdala is able use its vast and intimate connections to establish redundant neurosynaptic systems that may be able to use fear memories stored in the amygdala and other brain areas to mediate behavioural and autonomic fear responses. Thus, it is possible that the amygdala and its cortical and subcortical afferent and efferent pathways form emotional memory circuits that are involved in acquisition, expression and long-term storage of fear memories and classically conditioned fear (LeDoux, 1993; LeDoux, 1996; LeDoux, 2000; LeDoux 2002; LeDoux, and Muller, 1997).

5.4: Lesioning of the Amygdala or its Neurons Prevent the Acquisition and Expression of Pavlovian Conditioned Fear and Generally Disrupt Aversively Motivated Learning

Despite the minor disparities that arise from time to time between some scientists, there is general consensus that the certain key nuclei of the amygdala (i.e. lateral, basolateral and central) are involved in the acquisition and/or expression of conditioned fear (Davis, 1992a,b,c; 1995; 1997; 2000; LeDoux, et al., 1990; LeDoux, 1993; Helmstetter, 1992a,b;

Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999; Maren, 1996; 1999; 2001). Research indicates that conditioned fear acquisition or its expression is blocked by electrolytic and chemical lesions of the basolateral or central nucleus of the amygdala (Hitchcock and Davis, 1986; 1987; Davis, 1992: LeDoux et al., 1990: LeDoux, 1992; Sananes and Davis, 1992; Campeau and Davis, 1995a; Lee, Walker, and Davis, 1996b; Walker and Davis, 2000). For example, electrolytic lesions of the basolateral and central amygdaloid nucleus block the expression of FPS in rats when either an auditory or visual cue is used as a conditioned stimulus (Hitchcock and Davis, 1987; Hitchcock and Davis 1986; Kim and Davis, 1993). Similarly, Campeau and Davis (1995a) demonstrated that pre-training and post-training electrolytic or chemical lesions of the central or basolateral nucleus blocked FPS in rats trained concurrently using an auditory and visual conditioned stimulus.

Furthermore, fibre sparing NMDA neurotoxic lesions made prior to Pavlovian fear conditioning or three days after fear training completely blocked FPS in rats (Sananes and Davis, 1992), indicating that neurons in the basolateral nucleus of the amygdala are crucial for fear acquisition and expression. In addition, fibre sparing NMDA lesions of the BLA were shown to totally block the expression of FPS when lesions were made either 6 or 30 days after fear conditioning (Lee, Walker and Davis, 1996b) and the absence of a temporal gradient in this particular study seems congruent with the results obtained from several other studies (Maren, Aharonov, and Fanselow, 1996a; Kim and Davis, 1993a; 1993b; Cousins and Otto, 1998) that have been used to examine conditional fear. Also, Kim and Davis (1993a) reported that pretraining lesions of the basolateral and central amygdaloid nuclei prevents rats from acquiring FPS even if numerous Pavlovian fear conditioning trials are administered over a period of several days. More recently, experimental studies carried out on mice revealed that lesions of the BLA blocked the expression of FPS in these animals when both auditory and visual conditioned stimuli were used (Heldt, et al., 2000), a result that is very consistent with much of the earlier work carried out on the rat (Hitchcock and Davis, 1986; 1987; Sananes and Davis, 1992; Campeau and Davis, 1995a). On the whole, all of the studies discussed in this section (Lee, et al., 1996b; Maren, et al., 1996; Kim and Davis, 1993a,b; Cousins and Otto, 1998) seem to indicate that the amygdala may be a site that where long-term fear memories are permanently stored.

Phaseolus vulgaris-leucoagglutinin (PHA-L) anterograde and Fluoro-Gold retrograde tracing studies as well as other similar tracing techniques demonstrate that the caudal ventral amygdalofugal pathway forms a direct efferent pathway from the central nucleus of the amygdala to the startle circuit at the level of the reticularis pontis caudalis (Rosen, Hitchcock, Sananes, Miserendino, and Davis, 1991; Krettek and Price, 1978a; Post and Mai, 1980). Electrophysiological and extensive lesioning studies indicate that the acoustic startle response is most likely mediated by large cochlear root neurons projecting to the reticularis pontis caudalis and to spinal cord motor neurons (Lee, et al., 1996a). Thus, electrolytic lesions of the CeA or the caudal ventral amygdalofugal pathway at various levels as it travels to the brain stem startle circuit at the level of the reticularis pontis caudalis (RPC) have been shown to block FPS and the enhancement of startle normally observed after a brief series of footshocks (Hitchcock and Davis, 1991; Hitchcock et al., 1989). This research indicates that amygdaloid efferent reaching the RPC are necessary for the expression of FPS.

As was mentioned above, Kim and Davis (1993a) found that pretraining electrolytic lesions of the basolateral and central amygdala prevented rats from acquiring or expressing a FPS response even when extensive training was provided. Similarly, lesions of the central amygdaloid nucleus completely blocked the expression of FPS in rats even though these animals had been exposed to an extensive fear conditioning regime (i.e. 64 light + footshock trials), however it is interesting to note that these lesions did not prevent rats from reacquiring FPS when they were given extensive retraining (Kim and Davis, 1993a). Based on these results, Kim and Davis (1993a) stated that while the amygdala is absolutely crucial to fear learning there remains the possibility that reacquisition and expression of conditioned fear may be mediated by a secondary brain region once the amygdala has been damaged. It was hypothesised that the amygdala through its ascending efferent pathways could produce functional changes in a secondary brain area that also has connections to the midbrain startle circuit and that this likely explains why rats reacquire FPS in spite of amygdala damage (Kim and Davis, 1993a).

Although Kim and Davis (1993a) did not speculate on the exact brain region that may be involved in mediating the reacquisition of FPS after the amygdala has been damaged, it is quite conceivable that the BNST is a likely candidate that may take over this function.

This seems logical since the BNST sends projections to nuclei in the midbrain startle circuit (see Alheid, et al., 1995; Chapter 4 section 4.5) and since extensive and long-term training and retraining regimes like one used by Kim and Davis (1993a) tend to result in long-term sensitized acoustic startle responding, an effect that can be blocked by lesions of the BNST (Gewirtz and Davis, 1998). Thus, a long-term pre-amygdaloid-lesion training regime (i.e. 6 days of 16 light + shock trials) like the one used by Kim and Davis (1993a) may have permitted the amygdala to build redundancy into the fear system by passing on CS-UCS sensory information to neurons in the BNST that would then enable them to take over fear acquisition and expression functions if the amygdala sustained damage (Kim and Davis, 1993a). With this in mind, it is interesting to point out that the reacquisition of FPS can reportedly be blocked by lesions of the caudal ventral amygdalofugal pathway, but not by lesions of the central amygdaloid nucleus (see, Kim and Davis, 1993a,b). This indicates that the BNST which is known to send projections via the caudal ventral amygdalofugal pathway and have a near identical neurochemical and cellular profile to the amygdala is well suited to take over fear learning functions especially if its cells were sensitised by the amygdala before the amygdala sustained any damage from lesioning. In any event, the above results highlight the importance of the basolateral and central amygdaloid regions in conditioned fear learning and fear expression.

The acquisition of conditioned defensive freezing to both cue and contextual CS is disrupted by lesions primarily confined to the basolateral amygdaloid complex, whereas lesions of the dorsal hippocampus block contextual fear conditioning (Phillips and LeDoux, 1992). Also, a combined electrolytic and NMDA lesioning technique employed by Goosens and Maren (2001) demonstrated that lesions of the central, lateral and anterior basal nuclei blocked the acquisition of defensive freezing to a specific CS (i.e. tone), whereas lesions confined to the most posterior regions of the basal amygdaloid nucleus did not. In this particular study, the acquisition of contextual fear was also blocked by lesions to these amygdalar nuclei and there was a slight effect of laterality which seemed to indicate that amygdala lesions restricted to the right hemisphere generally produced more deficits to contextual fear learning, however this effect was quite small (Goosen and Maren, 2001). Moreover, electrolytic lesions confined to the lateral amygdaloid nucleus disrupted both the acquisition of defensive freezing and the CS-induced suppression of drinking

behaviour and caused a significant reduction in the conditioned elevation of blood pressure normally observed following exposure to a conditioned fear stimulus (LeDoux, Cicchetti, Xagoraris, and Romanski, 1990).

In addition, Iwata, et al., (1986) employed a disconnection lesioning technique that involved electrolytically lesioning the medial geniculate body (a major thalamic nuclei sending auditory and somatosensory information to the amygdala and caudate putamen) in one hemisphere while carrying out ibotenic acid lesions of neurons in the lateral amygdala, central nucleus of the amygdala and caudate putamen in the contralateral hemisphere which makes up the projection field of the medial geniculate nucleus. The results of this experiment demonstrated that destruction of neurons in the lateral and central nucleus of the amygdala, but not in the caudate putamen, caused a significant reduction in conditioned arterial blood pressure and defensive freezing (Iwata, et al., 1986). These experimental outcomes imply that intrinsic neurons in the subcortical field innervated by the medial geniculate nucleus of the thalamus, which includes the lateral and central nucleus of the amygdala, appear to be necessary for mediating fear conditioning and associative fear learning. In a similar manner, Young and Leaton (1996) demonstrated that defensive freezing, phasic heart rate accelerations, and tonic decreases in heart rate in response to aversive acoustic stimuli could all be blocked by lesions of the central nucleus of the amygdala.

5.5: Amygdala Lesion Block Several Different Kinds of Pavlovian Fear Responses

5.51: The Impact of Amygdala Lesions on Conditioned Hypoalgesia

Several other Pavlovian conditioned fear responses are blocked by amygdala lesioning or experimental manipulations carried out on this limbic structure. Conditioned hypoalgesia has been used in this regard. Conditioned hypoalgesia, is defined as the suppression of a stereotyped behavioural reaction to painful stimuli (i.e. paw lifting or licking in response to formalin injections made into the paw and/or tail flicking to avoid a radiant heat source) in the presence of a cue or cues previously paired with an aversive footshock (Watkins and Mayer, 1982; Fanselow, 1986b; Fanselow and Helmstetter, 1988; Helmstetter, 1992a; Helmstetter and Bellgowan, 1993). Conditioned hypoalgesia

responding is blocked by both electrolytic and ibotenic acid lesions of the central and basolateral amygdaloid region (Helmstetter, 1992a). In most cases, fear conditioned animals will suppress behavioural responses produced by pain when presented with either contextual or conditioned cues that predict the onset of an aversive event (i.e. footshock) and sham control rats in a study conducted by Helmstetter (1992a) exhibited both conditioned defensive freezing and conditioned hypoalgesia, whereas amygdala lesioned rats displayed significant deficits in both conditioned fear responses (Helmstetter, 1992a). Although both chemically induced lesions or electrolytic lesions of the basolateral and central amygdaloid regions blocked the acquisition and expression of this conditioned fear response they did not interfere with normal responses to painful stimuli as lesioned and sham animals did not differ significantly on baseline levels of formalin-induced paw lifting or licking (Helmstetter, 1992a). In a similar fashion, electrolytic lesions of the basolateral and much of the central amygdaloid nuclei also blocked conditioned hypoalgesia in a tail flick behavioural test when a specific cue that predicted the onset of footshock was presented to the group of lesioned rats, however baseline reactivity to the painful thermal stimulus was not altered by these lesions (Helmstetter and Bellgowan, 1993). Furthermore, direct infusions of diazepam into the BLA was shown to block both conditioned hypoalgesia and conditioned defensive freezing (Helmstetter, 1993) indicating that benzodiazepine receptor activation in the basolateral amygdaloid nucleus leads to significant anxiolytic affects that serve to reduce conditioned fear expression. This result is consistent with the work of Harris and Westbrook, (1994; 1995) who found that inhibiting amygdaloid neurons with benzodiazepine drugs or opioid compounds (i.e. morphine) significantly hampered conditioned analgesia.

Research has highlighted the importance of opioid receptors in the amygdala in fear learning and conditioned hypoalgesia (Gallagher and Kapp, 1978; Gallagher, Kapp, McNall and Pascoe, 1981; Good and Westbrook, 1995) and several lines of research seem to indicate that conditioned hypoalgesia is probably mediated by central amygdaloid projections to opioid-sensitive neurons in the ventrolateral PAG (De Olmos, et al., 1985; Gray and Magnuson, 1992; Basbaum and Fields, 1984; Watkins, Wiertelak and Maier, 1993) a midbrain region that is well known for its role in conditioned hypoalgesia, conditioned defensive freezing and pain control (Basbaum and Fields, 1984; LeDoux, et al.,

1988; Helmstetter and Landeira-Fernandez, 1990; Beitz, 1995). First of all, the amygdala and ventrolateral PAG are intimately connected to each other by several reciprocal pathways (Rizvi, Ennis, Behbehani and Shipley, 1991) and many projections from the central amygdaloid nucleus innervate ventrolateral portions of the PAG or pass through this region on route to the pons, rostral medulla, and lower brainstem regions (Hopkins and Holstege, 1978; Bandler,, McVulloch and Dreher, 1985; Gray and Magnuson, 1992; Rizvi, Ennis, Behbehani and Shipley, 1991; Wallace, et al., 1992). Second, electrically or chemically-induced stimulation of both the basolateral and central amygdaloid nuclei have been reported to produce analgesia in rats (Helmstetter, Bellgowan and Tershner, 1993; Kalivas, Gau, Nemeroff and Prange, 1982; Klamt and Pradi, 1991; Mena, Mathur and Nayar, 1995; Oliverira and Prado, 1994) and lesions of the ventrolateral PAG or pharmacological manipulation of opioid receptors in the PAG with naltrexone (an opioid receptor antagonist) has been shown to block the expression of conditioned hypoalgesia (Kinscheck, Watkins, and Mayer, 1984; Tershner and Helmstetter, 1992; Helmstetter and Landeira-Fernandez, 1990).

Third, most projections arising from the central amygdaloid nucleus have been shown to terminate in cellular columns of the PAG that are known to produce analgesia (Hopkins and Holstege, 1978; Rizvi, et al., 1991), and morphine infusions into the rostral portions of the basolateral and central amygdaloid nuclei have been shown to block the acquisition and expression of conditioned fear and hypoalgesia in rats (Good and Westbrook, 1995). It is speculated that this effect occur through the activation of opioid amygdaloid receptors that in turn act to suppress amygdaloid output to the PAG (Good and Westbrook, 1995). Indeed, morphine microinfusions into the amygdala reportedly caused a dose dependent blockade of conditioned hypoalgesia as the latency to initiate formalin induced paw licking was significantly shorter in rats that received high doses of morphine (4.0 μg and 8.0 μg) than in rats that received either saline or 2.0 μgs of morphine. Additionally, the latency to step-down off a hot plate apparatus that was previously paired with a painful radiant heat source was also significantly reduced in rats that were pre-treated with intra-amygdalar morphine infusions (Good and Westbrook, 1995). This finding indicates that rats treated with morphine prior to conditioning failed to acquire conditioned fear and hypoalgesia and

as a consequence could not express fear or hypoalgesia when re-exposed to the salient fear eliciting cue (i.e. the hot plate apparatus) (see Good and Westbrook, 1995).

Research evidence seems to indicate that amygdaloidal opioid receptors are involved in mediating fear learning and conditioned hypoalgesia since intra-amygdalar naloxone injections (an opioid receptor antagonist) reverses the impairments on the acquisition of conditioned hypoalgesia caused by morphine microinfusions made into the amygdala (Good and Westbrook, 1995). This finding is consistent with earlier scientific efforts that have revealed that immediate posttraining intra-amygdaloid infusions of opioid receptor agonists that act on the mu receptor complex prevent autonomic and behavioural expressions of fear from emerging on successive testing sessions (Gallagher and Kapp 1978; Gallagher, et al., 1981). Finally, experimental work obtained from several laboratories has demonstrated that the expression of conditioned hypoalgesia relies heavily on the functional integrity of the amygdala and various other brain regions such as the ventrolateral PAG, rostroventral medulla, nucleus reticularis paragigantocellularis, and dorsolateral funiculus that are all key components in a descending brainstem antinociceptive neural pathway responsible for the hypoalgesia that typically occurs after opioid microinfusions, electrical stimulation, and exposure to conditioned and unconditioned stressors (Helmstetter and Landeria-Fernadez, 1990; Watkins, Young, Kinscheck and Mayer, 1983; Kinscheck, Watkins and Mayer, 1984; Watkins, Cobelli and Mayer, 1982; Watkins and Mayer, 1982; Helmstetter, 1992b; Good and Helmstetter and Bellgowan, 1993; Good and Westbrook, 1995).

As mentioned earlier, the central amygdaloid nucleus has been shown to project to opioid-sensitive neurons located in the ventrolateral PAG that are involved in conditioned hypoalgesia and other forms of antinociception (Basbaum and Fields, 1984; Rizvi, et al., 1991) and many of these amygdaloid projections as well as descending projections arising out of the ventrolateral PAG ultimately reach ventral portions of the rostral medulla at the level of the reticularis paragigantocellularis (Basbaum and Fields, 1979; Beitz, Mullett and Weiner, 1983). Neurons in the rostral medulla then send descending projections via the dorsolateral funiculus to innervate cells in the dorsal horn of the spinal cord (Basbaum and Fields, 1978; Basbaum, and Fields, 1979; Basbaum and Fields, 1984; Rizvi, et al., 1991) and lesions of either the ventral rostral medulla or the dorsal funiculus have been shown to

block the expression of conditioned hypoalgesia (Tershner and Helmstetter, 1992; Watkins, Cobelli and Mayer, 1982). Thus, the amygdala and its efferent pathway to the ventrolateral PAG and ventral aspects of the rostral medulla that end up terminating in the superficial cellular layers of the dorsal horn likely makes up a neural pathway that is involved in the expression of conditioned hypoalgesia.

On the other hand, the acquisition of conditioned hypoalgesia likely depends on the integrative actions of the basolateral amygdaloid complex as either lesioning or pharmacological manipulation of this area with NMDA receptor antagonists (i.e. AP5) has been shown to prevent CS-UCS fear learning across a number of different behavioural paradigms (Sananes and Campbell, 1989; Miserendino, Sananes, Melia and Davis, 1990; Sananes, and Davis, 1992; Fanselow and Kim, 1994; Campeau and Davis, 1992; Hatfield and Gallagher, 1995; Ferry, Sander, and Di Scala, 1995; Maren, Aharanov and Fanselow, 1996; Cousens and Otto, 1998; for reviews also see Davis, 1992; 1995; 2000; LeDoux, 1993; 1996; 2000; Fendt and Fanselow, 1999). For example, excitotoxic acid lesions of the basolateral amygdaloid nucleus or infusion of NMDA receptor antagonists (i.e. AP5) into this limbic region significantly impaired the acquisition of taste-potentiated odour aversion, whilst leaving taste aversions and olfactory perception intact (Ferry, et al., 1995; Hatfield and Gallagher, 1995). These results imply that the amygdala may be involved in linking olfactory and gustatory cues to aversive events in order to help an animal avoid potentially harmful situations or poisonous substances during times of food gathering.

5.52: The Role of the Amygdala in Aversion Learning

In most instances, rats develop a strong aversion to an olfactory cue that is paired with both a gustatory stimulus (i.e. taste stimulus) and delayed illness (Rusiniak, Hankins, Garcia and Brett, 1979; Durlach and Rescorla, 1980; Palmerino, Rusiniak, and Garcia, 1980). However, presentation of an odour cue with a delayed illness produces no aversion to the olfactory cue and this type of pairing generally leads to the development of taste aversions only (Rusiniak, et al., 1979; Durlach and Rescorla, 1980; Palmerino, et al,, 1980; Hatfield, Graham, and Gallagher, 1992). Research has been able to show that discrete electrolytic or neurotoxic NMDA lesions of the BLA that typically block taste potentiated odour aversion (TPOA) have no deleterious impact on conditioned taste aversion learning

(Bermudez-Rattoni, Grijalva, Kiefer, and Garcia, 1986; Hatfield, et al., 1992). There is some indication that the acquisition of TPOA seems to be specifically controlled by the BLA since lesioning of the central amygdaloid nucleus, caudate putamen and entorhinal cortex did not produce any detrimental effect on either TPOA or conditioned taste aversion learning (Ferry, et al., 1995).

On the whole, the above lesion results are generally consistent with pharmacological manipulations that temporarily inactivate the BLA. For example, infusion of either the $GABA_A$ receptor agonist muscimol or the local anaesthetic novocaine into the basolateral amygdaloid complex just prior to the acquisition training phase prevented learning of the TPOA response, and like the lesions, these infusions did not effect aversion to the taste cue (Ferry, et al., 1995; Bermudez-Rattoni, Rusiniak and Garcia, 1983). Additionally, immediate posttraining intra-amygdala infusions of muscimol, but not novocaine, were found to impair TPOA when animals were subsequently tested for the acquisition of this aversively motivated response (Bermudez-Rattoni, et al., 1983; Ferry, et al., 1995). In contrast, infusions of muscimol into the dense GABAergic fields of the BLA (Pitkänen and Amaral, 1994) long after the animals had been trained and just prior to final testing did not effect the expression of TPOA (Ferry, et al., 1995) suggesting that the BLA is not required for the expression of this conditioned response. This notion seems to make sense as infusions of the NMDA antagonist AP5 into the BLA blocked the acquisition of TPOA but had no impact on its expression (Hatfield and Gallagher, 1995).

5.53: The Amygdala and Olfactory Heart Rate Conditioning

Olfactory cues can be used to elevate cardiovascular responses (i.e. heart rate) provided that an aversive event (i.e. shock) is repeatedly paired with the desired olfactory stimulus during classical conditioning (Sananes, Gaddy, and Campbell, 1988; Sananes and Campbell, 1989). Thus, like the TPOA paradigm, the olfactory heart rate conditioning paradigm also incorporates olfactory cues during training in order to potentiate a particular response (Rusiniak, et al, 1979; Durlach and Rescorla, 1980; Palmerino, et al., 1980; Hatfield, Graham, and Gallagher, 1992; Sananes, et al., 1988; Sananes and Campbell, 1989). Research data gathered from this paradigm demonstrates that electrolytic or ibotenic lesions of the central nucleus of the amygdala disrupt fear conditioning in rat pups

when a specific olfactory cue is paired with a mild subcutaneous shock during training (Sananes and Campbell, 1989). The rat pups in the Sananes and Campbell (1989) study exhibited profound impairments in developing fear-induced increases in heart rate in response to the presentation of the olfactory CS. However, the decelerations in heart rate that are indicative of a normal orienting response to the olfactory cue and the unconditioned increase in heart rate in responses to the aversive subcutaneous shock were not affected by these amygdala lesions (Sananes and Campbell, 1989). Taken together, the above findings are congruent with numerous research studies that have demonstrated that the amygdala is an essential limbic brain region that is involved in fear-mediated heart rate conditioning when innocuous auditory stimuli are paired with aversive events (Kapp, Frysinger, Gallagher and Haselton, 1979; Applegate, et al., 1982; Gallagher, Kapp, Frysinger, and Rapp, 1980; Gentile, Jarrell, Teich, McCabe and Schneiderman, 1986).

More recently, Cousens and Otto (1998) have shown that excitotoxic NMDA lesions of the basolateral amygdaloid nucleus made prior to olfactory fear conditioning in a defensive freezing paradigm eliminated immediate postshock freezing and subsequent conditioned defensive freezing to both the olfactory CS and the training context where the animals received olfactory cue + footshock Pavlovian fear conditioning trials. These data seem to strongly support the notion that neurons in the basolateral amygdaloid complex are important for forming CS-UCS associations that occur during Pavlovian fear conditioning. Furthermore, NMDA lesioning of the BLA either one or fifteen days after fear conditioning (six odour + shock trials) effectively attenuated both odour-induced and contextual freezing responses, indicating that the neurons in the BLA have an enduring role in fear memory retrieval processes and in the expression of conditioned freezing behaviour that is triggered by olfactory and contextual stimuli previously paired with footshock (Cousens and Otto, 1998). The results obtained from the lesion studies carried out by Cousens and Otto (1998) are highly consistent with earlier research studies that have examined the role of the BLA in the acquisition and expression of defensive freezing behaviour when specific auditory stimuli are contiguously and repeatedly paired with aversive footshocks (see Maren, et al., 1996a).

5.54: Lesion Studies Demonstrating the Involvement of the Amygdala in Conditioned Fear and Defensive Freezing Behaviour

A wealth of research using the defensive freezing paradigm to assess conditional fear in animals indicates that lesions of the basolateral and central amygdaloid nucleus block fear acquisition and expression in laboratory animals (Iwata, LeDoux, Meeley, Arneric, and Reis, 1986; LeDoux, et al., 1988; Phillips and LeDoux, 1992; Goosens and Maren, 2001; Helmstetter, 1992b; Maren, Aharonov and Fanselow, 1996a). More specifically, LeDoux, and colleagues (1988) demonstrated that discrete lesions confined to the lateral and dorsal borders of the basolateral amygdaloid nuclei greatly reduced both the acquisition of cue specific defensive freezing and conditioned increases in mean arterial blood pressure that are usually evoked by a fear eliciting CS. A second experiment conducted in this same study, also revealed that lesions to the lateral amygdala interfered with the conditioned suppression of drinking (LeDoux, et al., 1988). Typically, rats with lateral amygdala lesions spent more time drinking and less time defensively freezing in the presence of a CS previously paired with footshock than sham controls indicating that lesions made prior to fear conditioning interfered with fear learning in this paradigm (LeDoux, et al., 1988).

In addition, lesion studies conducted by Maren and associates (1996a) and various other researchers (Kim and Davis, 1993b; Lee, Walker and Davis, 1996; Cousens and Otto, 1998) seem to indicate that the basolateral and central amygdaloid nuclei have a long-term role in the expression of conditioned fear. For example, excitotoxic NMDA lesions of the basolateral amygdaloid nucleus seven days prior to fear conditioning or one, fourteen, or twenty eight days after Pavlovian fear conditioning completely blocked the expression of conditioned defensive freezing behaviour to the acoustic CS and to the contextual cues of the testing/training apparatus (Maren, et al., 1996a). More specifically, BLA neurotoxic lesions made seven days prior to fear conditioning prevented lesioned rats from acquiring and then later expressing fear as measured by the behavioural freezing response (Maren, et al., 1996a). Similarly, BLA lesions made at various intervals after fear training were effective in blocking fear expression and this expression deficit seemed to linger even after an extensive retraining regime was administered to these animals (Maren, et al., 1996a). In stark contrast, sham operated controls exhibited robust freezing to both contextual and

acoustic conditioned stimuli during the acquisition training and testing phase and at all training-to-lesion intervals (Maren, et al., 1996a).

Additional evidence indicating an enduring role for the basolateral amygdaloid nucleus neurons in fear acquisition, fear memory consolidation and fear expression comes from both the defensive freezing and FPS paradigms. For example, neurotoxic lesions of the BLA have been shown to cause marked impairments in fear learning and fear memory formation as measured by defensive freezing when an auditory CS was employed during Pavlovian fear conditioning (Maren, 1999b). In particular, a majority of basolateral amygdaloid lesioned rats in a study conducted by Maren (1999b) failed to exhibit fear to the auditory CS or have any savings of conditional fear when compared to control rats despite the fact that the lesioned animals received extensive training. As a consequence, these amygdala-lesioned rats exhibited profound difficulties in reacquiring conditioned defensive freezing even when given numerous CS-UCS retraining trials, whereas control rats did not (Maren, 1999b).

Similarly, Maren (2001) found no evidence indicating a savings of contextual fear memories in rats that received posttraining NMDA lesions of the basolateral amygdaloid nucleus and rats with these lesions tended to display profound impairments in conditional fear despite receiving extensive fear training (e.g. 75 trials) prior to the lesion. This finding is generally consistent with previous research that has shown that extensive overtraining does not result in savings of either auditory or contextual conditional fear in animals that have been administered posttraining lesions of the BLA (Maren, 1998; 1999b). Typically, NMDA-lesioned rats took significantly longer to initiate defensive freezing (i.e. higher latencies) than the sham controls, displayed less bouts of freezing during testing, exhibited a lower duration of defensive freezing and frequently froze less than control animals (Maren, 2001). More specifically, sham rats that received no fear training prior to surgery acquired conditioned fear after retraining sessions at a much faster rate than rats that either received seventy-five conditioning or no fear conditioning trials prior to NMDA lesioning (Maren, 2001) indicating that there were no fear memory savings in rats that received seventy-five conditioning trials prior to lesioning. This finding suggests that posttraining NMDA lesions of the BLA most likely erase CS-UCS associations that are formed in the amygdala during fear conditioning because even when rats received extensive

contextual fear conditioning prior to NMDA lesioning, it did not help them reacquire contextual fear faster than sham animals that received no training prior to surgery (Maren, 2001). Although Maren (2001) did report that extensive retraining of basolateral amygdaloid lesioned animals does eventually lead to the acquisition or reacquisition of contextual conditioned fear, presumably by recruiting other neural systems that may be involved in contextual fear conditioning (e.g. dorsal hippocampus; see Phillips and LeDoux, 1992), he did indicate that even this takes a long time to occur in animals that have received NMDA lesions of the amygdala and that this effect does not seem to occur when sensory specific auditory cues are used during fear training and testing (Maren, 2001; Maren, 1999b).

5.55: Fear –Potentiated Startle is blocked by Lesions of the Amygdala or Regions that Provide Sensory Input to the Amygdala

It is important to note that very similar deficits in fear acquisition and expression following electrolytic or chemical lesioning of the BLA have been reported by studies that use the FPS paradigm to assess levels of conditioned fear in rats. For example, NMDA or electrolytic lesions of the BLA made 6 or 30 days after fear conditioning were equally effective in blocking FPS in laboratory rats (Kim and Davis, 1993b; Lee, Walker, and Davis, 1996b) and it is important to note that the lack of a temporal gradient of retrograde amnesia in rats administered amygdala lesions is congruous with the work of Maren and colleagues (1996a) who demonstrated that NMDA amygdala lesions made either 7 days before or 1, 14, or 28 days after Pavlovian fear training completely attenuated fear-induced defensive freezing. Furthermore, scientific evidence obtained from lesion and anatomical tracing studies indicates that Pavlovian conditioned fear acquisition, retrieval, and expression likely relies on the functional integrity of discrete thalamo-amygdaloid and thalamo-cortico-amygdaloid neural circuits that transmit auditory, visual, and somatosensory information to the lateral and basolateral nuclei of the amygdala and not on input arising from primary sensory cortical regions (Romanski and LeDoux, 1992a,b; Campeau and Davis, 1995b; Falls and Davis, 1993; Shi and Davis, 1999; Shi and Davis, 2001; Doran and LeDoux, 1999; LeDoux, et al., 1990a, b; Shi and Cassell, 1998; 1999; Linke, De Lima, Schwegler, and Pape, 1999; Linke, Braune, Schwegler, 2000). For

example, pretraining lesions of the lateral and basolateral amygdaloid nuclei block the acquisition and expression of FPS in rats (Sananes and Davis, 1992; Kim and Davis, 1993a; Campeau and Davis, 1995a). In stark contrast, large lesions of either the occipital lobe or the primary auditory cortex (Te1) did not prevent rats from acquiring the FPS response (Falls and Davis, 1993; Campeau and Davis, 1995b). In addition, posttraining lesions of the amygdala (i.e. basolateral and central nuclei) or the visual thalamus (i.e. the lateral geniculate body and the lateral posterior nucleus) have been shown to completely disrupt the expression of FPS to a visual CS (Sananes and Davis, 1992; Campeau and Davis, 1995a; Shi and Davis, 2001), whereas posttraining lesions of the occipital, frontal and medial prefrontal cortices failed to do so (Rosen, et al., 1992; Falls and Davis, 1993). In a similar fashion, posttraining lesions of the central amygdaloid nucleus and pretraining lesions of the basolateral nucleus of the amygdala blocked the expression and acquisition of FPS respectively when an auditory CS was used, as did pre- and post training lesions of the auditory thalamus (i.e. dorsal and ventral subdivisions of the medial thalamic nucleus; MGV/MGD) (Sananes and Davis, 1992; Campeau and Davis, 1995a; 1995b).

Specifically, posttraining electrolytic and neurotoxic lesions of the central amygdaloid nucleus and pre- and post training electrolytic and NMDA lesions of the basolateral amygdaloid complex was observed to abolished FPS in rats when both auditory and visual cues were integrated into the fear training and fear testing experimental protocol (Campeau and Davis, 1995a). Also, pre- and post training electrolytic and chemical lesions of the auditory thalamus (i.e. MGV/MGD) which innervates the lateral amygdaloid nucleus has been reported to disrupt FPS to auditory conditioned cues (Campeau and Davis, 1995b) and recent work by Shi and Davis (2001) has demonstrated that either posttraining lesioning or functional inactivation of the visual thalamus (i.e. lateral posterior nucleus and lateral geniculate body of the thalamus) with the AMPA receptor antagonist NBQX attenuates the expression of FPS in rats. Conversely, lesions of the primary auditory and visual cortical regions have for the most part failed to produce deficits in the acquisition and expression of FPS (see Falls and Davis, 1993; Campeau and Davis, 1995b).

Despite this fact, posttraining lesions of the multimodal sensory processing regions such as the PRh and Te3 which innervate the lateral amygdala and receive input from the visual, auditory, and sensory thalamic nuclei (Doran and LeDoux, 1999; Linke, et al., 1999; 2000;

LeDoux, 1990a; Shi and Cassell, 1999; Shi and Davis, 1996; 2001 Romanski, et al., 1993; McDonald, 1998) block the expression of FPS to auditory and visual conditioned stimuli (Rosen, et al., 1992; Campeau and Davis, 1995b; also see Shi and Davis, 1999; 2001). This indicates that thalamo-cortico-amygdalar pathways and possibly the PRh may be involved in facilitating fear memory retrieval via its projections to the lateral and BLA. However, it is important to note that large NMDA lesions of the entire PRh failed to disrupt the acquisition of FPS which indicates that direct thalamo-amygdaloid circuits may be sufficient to stimulate Pavlovian fear learning even if thalamo-cortico-amygdaloid neural systems are damaged (Romanski and LeDoux 1992a,b; Campeau and Davis, 1995b). The negligible impact on the acquisition of FPS made by pretraining PRh lesions is highly consistent with the work of Romanski and LeDoux (1992b) who demonstrated that pretraining lesions of this particular cortical region did not interfere with the acquisition of auditory fear conditioning in a defensive freezing paradigm. Taken together, the lesion studies mentioned above suggest that intact thalamo-amygdalar or indirect thalamo-cortico-amygdaloid pathways are essential for providing the amygdala with the multimodal sensory information that makes Pavlovian fear learning and expression possible. Thus, on the basis of the lesion studies discussed above, it appears as though the amygdala may be a key site where CS-UCS fear associations are formed and where fear memories are permanently stored.

5.56: The Amygdala's Contribution to Fear Learning and Memory Storage is Still Vigorously Debated: Is the Amygdala a Site Where CS-UCS Fear Memories are Permanently Stored?

Despite all the research evidence highlighting the important role of the basolateral amygdaloid nucleus in fear acquisition and fear-motivated learning some researchers (McGaugh, Mesches, Cahill, Parent, Coleman, Mesches, and Salinas, 1995; Cahill and McGaugh, 1998; Cahill, Weinberger, Roozendaal and McGaugh, 1999; Weinberger, 1998) still maintain that the amygdala plays only a temporary role in the consolidation of fear memories. These research scientists argue that the majority of aversive memories are neither encoded nor stored in the amygdala and that most fear memories are stored elsewhere (McGaugh, et al., 1995; Cahill and McGaugh, 1998; Cahill, Weinberger,

Roozendaal and McGaugh, 1999; Weinberger, 1998). In essence, McGaugh and his associates (see McGaugh, et al., 1995; Packard, Williams, Cahill and McGaugh, 1995) tend to promote the view that the amygdala modulates plasticity in other brain regions.

Thus, according to this alternative view, the basolateral complex and portions of the central amygdaloid nucleus do not have an enduring role in encoding, storing or retrieving fear memories. Rather the amygdala is alleged to play a modulatory role by facilitating the storage of fear memories in other brain areas and then at appropriate times the amygdala is directed into retrieving these memories in order to respond to sensory cues that signal impending danger (McGaugh, et al., 1995; Cahill and McGaugh, 1998; Cahill, et al., 1999; Weinberger, 1998). For example, the proponents of this view contend that amygdala lesions do not attenuate fear-motivated inhibitory avoidance behaviour if they are made at 10 or more days after training has been completed (Liang, McGaugh, Martinez, Jensen, Vasquez and Messing, 1982; McGaugh, et al., 1995). These researchers also argue that extensive inhibitory avoidance training procedures generally mitigate the disruptive impact on fear-motivated behaviours when amygdaloid lesions are made after training (Parent, Tomaz and McGaugh, 1992; McGaugh, et al., 1995).

In fact research looking into the effects of amygdaloid lesions on inhibitory avoidance behaviour has shown that extensive inhibitory avoidance training (i.e. overtraining) does tend to reduce the memory impairments in inhibitory avoidance tasks caused by these posttraining lesions (Brady, Schreiner, Geller, and Kling, 1954; Horvath, 1963; Thatcher and Kimble, 1966; Parent, et al., 1992), which is consistent with the notion that the amygdala may modulate long-term memory processes elsewhere (McGaugh, et al., 1995; Packard, et al., 1995; Cahill and McGaugh, 1998; Cahill, et al., 1999). However, Davis and his research group (see Kim and Davis, 1993a,b; Lee, et al., 1996b) as well as other researchers (Maren, et al., 1996a; Fanselow and LeDoux, 1999) do make an important point by suggesting that the conditioned motor component of inhibitory avoidance behaviour but not the conditioned fear component may be what is responsible for instigating inhibitory avoidance behaviours after amygdaloid lesioning has occurred.

These researchers propose that the amygdala may only be momentarily involved in the consolidation of conditioned motor responses that are presumably stored in brain areas other than the amygdala (Kim and Davis, 1993a,b; Lee, et al., 1996b; Maren, et al., 1996a;

also see Liang, et al., 1982; McGaugh, 1989 in Kim and Davis, 1993b). As a result, what remains intact and is resistant to amygdaloid lesioning is the conditioned motor component that develops after extensive inhibitory training (Kim and Davis, 1993a,b; Lee, et al., 1996). Basically, this means that the survival of inhibitory avoidance in amygdala-lesioned animals may be due to the establishment of aversively conditioned motor responses after extensive avoidance training. In any event, it must be emphasised that a wealth of research evidence mentioned in earlier communications made in this thesis does seem to suggest that fear memories formed out of Pavlovian fear conditioning are stored within the confines of the amygdala on a permanent basis. This is consistent with the view that the amygdala is a malleable neural substrate or foundation upon which biochemical and molecular changes generated by classical fear conditioning can promote long-term synaptic modifications that make fear learning, storage, and expression possible (Davis, 1992a,b,c; Maren, 1999; Fanselow and LeDoux, 1999; LeDoux, 2000; Walker and Davis, 2000; Maren, 2003). Logically, the next topic that must be explored is how the amygdala accomplishes fear learning and memory consolidation at a neurosynaptic and molecular level. In other words, how are the CS-UCS fear associations that are established during Pavlovian fear conditioning and then measured behaviourally related to changes in synaptic strength and neurobiochemical functioning within the amygdala? The subject matter regarding how amygdaloid neural circuitry undergoes changes in synaptic efficacy during and after Pavlovian fear conditioning will be discussed in the next chapter.

Chapter 6

Amygdaloidal Emotional Fear Memory Circuits and their involvement in fear learning, fear memory storage, and Long-Term-Potentiation: The cellular, molecular, biochemical and synaptic mechanisms of fear conditioning and fear memory formation:

6.1: An Introduction to LTP and its Links to Learning and Memory

The notion that sensory information concerning biologically relevant stimuli is stored in the brain as changes in synaptic efficacy occurred well over one hundred years ago (Bliss and Collingridge, 1993; LeDoux, 2002). It is possible that this view may have had its early beginnings with Sechenov's ideas concerning how memory traces between mental processes and reflexive responses are formed and represented in the cortex. However, it is likely that today's comprehension of the neuroanatomical and neurochemical events underlying the increases in synaptic efficacy (i.e. Long-Term-Potentiation) began with Raymónd y Cajal (1852-1934) who demonstrated that networks of neurons were more than the mere tangled mass of cytoplasm, cells, and axons described by Golgi (Bliss and Cooingridge, 1993; Kolb and Whishaw, 1990). After observing the growth of axons as they migrated to their specified cellular targets, Cajal (1852-1934) came to the revolutionary conclusion that groups of neurons formed distinct and specialized neural networks (Cajal in Kolb and Whishaw, 1990) which communicate with each other at specialized terminal junctions called "synapses", a term devised by the great physiologist Charles Sherrington (1900; 1906 in Kolb and Whishaw, 1990).

Sherrington's research (1906; in Kolb and Whishaw, 1990) on reflex chaining and spatiotemporal patterns of neural activation commonly referred to as the functional integration of the nervous system combined superbly with Pavlov's (1927) studies into classical conditioning. This early work helped to develop and promote the view that when sensory stimuli in the environment are paired with innate reflexive responses they can come to be represented in the brain as spatial and temporal patterns of neural activity that can in and of themselves act as agents of synaptic change that are capable of supporting associative learning processes and memory formation (Bliss and Collingridge, 1993). Simply stated, the act of repeatedly pairing an innocuous stimulus (i.e. light or tone) with

aversive events (i.e. shock) was thought to energize pathways and promote synaptic changes in discrete brain areas that in turn would hold a memory trace of the conditioning experience that could, during appropriate situations, be reactivated to influence behavioural responses (Pavlov, 1927; Konorski, 1948; Hebb, 1949). Thus, the location of long-term storage which constitutes the engram of learning and memory was thought to reside in those brain areas that exhibit and maintain enhanced levels of synaptic transmission and/or activity-dependent changes in synaptic functioning (Bliss and Collingridge, 1993).

This notion of an engram for learning and memory emerging out of both activity-dependent changes in synaptic efficacy and increases in the levels of synaptic transmission was further refined by Hebb (1949) and Konorski (1948) who put forward the coincidence-detection rule that described the synaptic relationship that most likely developed between neurons during active periods (e.g. when CS were being paired with UCS). Simply put, this rule suggested that a synapse linking two cells is strengthened if both the presynaptic and postsynaptic cells are active at the same time or are repeatedly activated in such a manner where depolarization currents and action potentials in the presynaptic cell cause the release of chemical messengers which induce changes in the post synaptic cell (Hebb, 1949; Bliss and Collingridge, 1993; LeDoux, 2002).

Hebb (1949) further expanded on the coincidence-detection rule by suggesting that even weak synaptic contacts or signals between neurons can become strengthened if they are spatially or temporally paired with strong signals emanating from cells that share robust synaptic contacts. This pairing process also called "associativity" provides a cellular analogue of classical fear conditioning in that it represents the presynaptic and postsynaptic relationships that evolve or develop when a neutral stimulus (i.e. light) that initially causes weak synaptic activation is repeatedly paired with an aversive stimulus such as footshock which causes strong synaptic activation (Bliss and Collingridge, 1993; Schafe, Nader, Blair and LeDoux, 2001; LeDoux, 2002). Thus, over time, certain neuronal pathways are strengthened to the point where structural and biochemical changes in presynaptic and postsynaptic neurons serve to form a series of molecular-coincidence-detectors (MCD) that can act as biochemical/cellular circuits of learning and memory that are capable of interpreting and amplifying synaptic signals, deciphering biochemical messages, altering neuronal activity and influencing behaviour (Bliss and Collingridge, 1993). Although Hebb

(1949) provided an early elegant theory into how memories, including fear memories may be stored in the brain at a neurosynaptic level it took over two decades for the science to catch up with the theory and provide irrefutable experimental evidence that long-term enhancements of synaptic transmission can occur in certain brain regions.

The first modified synapses to be identified in the mammalian brain were the excitatory connections made by perforant path fibres impinging on granule cells in the hippocampal formation (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973). The enhancement in synaptic strength of this monosynaptic pathway was produced by brief trains of high-frequency stimulation (i.e. tentanic stimulation) that induced a quick and persistent increase in the efficiency of synaptic transmission. As a result, Long-Term-Potentiation (LTP) has been defined as an enhancement of synaptic efficacy produced by the high frequency stimulation of afferent pathways (Bliss and Lomo, 1973; Eccles, 1987; Teyler and DiScenna, 1987; Collingridge and Bliss, 1987; Brown, Chapman, Kairiss, and Keenan, 1988) and has been found to occur in several excitatory pathways in the hippocampus (Bliss and Lomo, 1973; also see Collingridge and Bliss, 1987; Kandel, 1997; Malenka and Nicoll, 1999; Kandel, et al., 2000) and in the lateral and BLA (Chapman, Kairiss, Keenan and Brown, 1990; Clugnet and LeDoux, 1990; Maren and Fanselow, 1995; Huang and Kandel, 1998; Weisskopf, Bauer, and LeDoux, 1999; Huang, Marin and Kandel, 2000; Bauer, et al., 2002).

LTP has been shown to be mediated by excitatory amino acid receptors in the hippocampus a brain region known to have a functional role in memory storage and retrieval processes (Dunwiddle, Madison, and Lynch, 1978; Collingridge, Kehl, and McLennan, 1983; Morris, Andersen, Lynch, and Baudry, 1986; Lynch and Baudry, 1984; Cotman, Monaghan, and Ganong, 1988; Reymann, Matthies, Schulzeck and Matthies, 1989) and a wealth of research seems to suggest that NMDA receptors located on the dendritic spines of postsynaptic neurons are involved in LTP induction and therefore may act as molecular coincidence detectors (Collingridge and Bliss, 1987; Bliss and Collingridge, 1993; Collingridge, Kehl, and McLennan, 1983; Coan, Saywood and Collingridge, 1987; Cotman, et al., 1988; Bashir, Tam, and Collingridge, 1990; Kandel, 2000; Kandel and Siegelbaum, 2000). For example, LTP induced by tentanic stimulation can generally be blocked by several types of NMDA receptor antagonists that have

different mechanisms of action such as 2-amino-5-phosphonopentanoic acid (AP5) that acts directly on the receptor complex at the glutamate binding site, MK-801 that binds to a receptor site localized within the open channel pore or 7-chlorokynurenic acid that interacts with the allosteric glycine binding site (Collingridge, et al., 1983; Coan, et al., 1987; Bashir, et al., 1990; Kandel, Schwartz and Jessell, 2000). Since LTP has been described as a neurobiological model of learning and memory it has been used extensively to define and better understand the hippocampal formation's role in mediating learning and memory processes (Eccles, 1987; Lynch and Baudry, 1984; Tyler and Discenna, 1987; Madison, Malenka and Nicoll, 1991).

In general, models that have investigated hippocampal LTP processes have established that the induction of LTP is mediated by the neurotransmitter glutamate and two major classes of ionotropic glutamatergic receptors which include NMDA and AMPA/kainate receptors and several subclasses of metabotropic glutamate receptors (mGluRs) (Madison, et al., 1991; Malenka and Nicoll, 1993; Bliss and Collingridge, 1993; Maren and Baudry, 1995; Kandel, 2000; Kandel and Siegelbaum, 2000 in Kandel, et al., 2000; Cotman, et al., 1988). It is hypothesised that many of these NMDA receptor channels increase their permeability to calcium during periods of cellular depolarization caused by tentanic stimulation (Tyler and Discenna, 1987; Cotman, et al., 1988; Madison, et al., 1991; Bliss and Collingridge, 1993; Kandel, et al., 2000 in Kandel, Schwartz and Jessell, 2000). Thus, it is highly likely that calcium entry into the postsynaptic cell via the NMDA receptor complex may play a prominent role in facilitating LTP induction (Bliss and Collingridge, 1993; Kandel, et al., 2000 in Kandel, Schwartz and Jessell, 2000). In this connection, it is noteworthy that pharmacological agents such as dantrolene that acts on the ryanodine receptor to suppress calcium release and thapsigargin that depletes intracellular calcium reserves have been shown to hinder LTP induction in the hippocampus (Bortolotto and Collingridge, 1993; Obenaus, Mody and Baimbridge, 1989, Harvey and Collingridge, 1992) indicating that calcium entry via the NMDA channel is a necessary step in the cascade of events that lead to LTP induction. This finding is consistent with the research demonstrating that intra-cellular administration of calcium chelator (i.e. a compound that interferes with calcium mediated biochemical processes in the postsynaptic cell) prevents LTP from occurring (Lynch, Larson, Kelso, Barrionueveno, and Schottler, 1993) as well as

the experimental evidence indicating that reductions in extracellular calcium levels near postsynaptic cells blocks LTP (Dunwiddie and Lynch, 1979). Recently, the molecular and biochemical processes that occur during LTP have begun to be clarified in greater detail in both the hippocampus (Kandel, 1997; Milner, Squire, and Kandel, 1998; Malenka and Nicoll, 1999) and the lateral nucleus of the amygdala (Brambilla, et al., 1997; Huang and Kandel, 1998; Huang, et al., 2000; Schafe, et al., 2000) and there is strong evidence to suggest that many of the biochemical events associated with LTP in the amygdala may have a profound impact on fear learning and fear memory consolidation (McKernan and Shinnick-Gallagher, 1997; Schafe, et al., 2001; Schafe, et al., 2000; Maren, 1999).

6.12: The Fundamental Properties of LTP and their link to classical conditioning

From a theoretical and practical standpoint, LTP has evolved into a well developed model that helps to describe how learning and memory formation occurs at a biochemical and molecular level in the mammalian brain. Since its experimental début in 1973, LTP has generally been characterized by three fundamental properties, these include; "cooperativity", "associativity", and "input specificity" (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). Cooperativity is used to operationally define the existence of intensity thresholds, patterns, and parameters that are required to be present in order for LTP to be successfully induced. For example, a weak stimulus signal triggered either by mild tentanic electrical stimulation or the presentation of innocuous sensory cues (i.e. light) often does not have the capacity to induce LTP because these types of events activate relatively few neuronal afferent pathways in a specified region. Thus, simply pairing two weak tentanic electrical currents or two innocuous sensory stimuli (e.g. light + mild auditory tones) in an animal's environment often will not result in LTP or any significant behavioural change that could be defined as classically conditioned learning. However, if a weak stimulus input or tetanus is simultaneously paired with a strong stimulus or occurs at the same time a strong stimulus is delivered through a separate but converging pathway, the weak stimulus input can be potentiated or enhanced to the point where it will activate neurons in much the same fashion as the strong input normally would. This process called "associativity" is really an exquisite example of a cellular analogue of classical conditioning because it provides an electrophysiological and molecular explanation of how a weak stimulus such as

a light, when repeatedly paired with a powerfully aversive stimulus (i.e. footshock) could cause biochemical alterations at the level of synapses in certain brain regions (e.g. amygdala) that in turn produce behavioural changes that are indicative of fear learning and fear memory consolidation. Hence, "associativity" makes it possible to link behavioural responses that occur during classical fear conditioning with changes in cellular activity, biochemistry and synaptic functioning (see Bliss and Collingridge, 1993; Maren, 1999; Schafe, et al., 2001).

The property of "input specificity" assumes that only synapses and cells that are active during the time of excitation (e.g. excitation induced by either tetanus or the pairing of a CS (i.e. tone) with the UCS footshock) will become more efficient in the long-term and thus will be able to influence future behaviour (see Bliss and Collingridge, 1993; Maren, 1999; Schafe, et al., 2001). In contrast, synapses and cells that are not active during tetanus or during the excitation caused by CS-UCS pairings will not exhibit any long-term changes and as such, will have limited impact on learning and behavioural responses. In fact, the property of "input specificity" seems to account well for what actually happens during Pavlovian fear conditioning. For instance, if a sensory specific cue that activates only one sensory modality is paired with an aversive UCS on a number of occasions, the presentation of the specific CS alone can not only influence the firing pattern and response latencies of amygdaloid neurons and but can also increase the level of conditioned fear displayed by the animal under investigation (Rogan, Staubli, and LeDoux, 1997; McKernan and Shinnick-Gallagher, 1997). In this connection it is noteworthy that Pavlovian fear conditioning can cause associative LTP to occur in the lateral and basolateral amygdaloid region (Rogan, et al., 1997; McKernan and Shinnick-Gallagher, 1997).

Perhaps one of the most ambitious scientific efforts over the past three decades has been to link together the biochemical events associated with artificial LTP induction that is normally observed in tissue samples gathered from brain slices with animal models of learning and memory consolidation that use live animals in experimental procedures that are designed to recruit brain regions that have traditionally been thought to be involved in mediating certain types of learning and memory consolidation functions (Bliss and Lómo, 1973; Chapman and Brown, 1988; Chapman, Kairiss, Keenan and Brown, 1990; Collingridge and Bliss, 1993; Schafe, et al., 2001; Kandel, et al., 2000). These efforts have

been undertaken in order to better unravel the complex molecular and biochemical cascades that occur during LTP when a live animal is subjected to Pavlovian conditioning tasks. Although many of the neurochemical events associated with LTP induction during fear learning and memory consolidation are numerous and complex, they can still be easily understood if they are distilled down into their individual parts.

First it is essential to understand that LTP and memory consolidation share a close relationship with each other since LTP, like memory consolidation, can be divided into distinct temporal phases (Davis and Squire, 1984; Kandel, 1997; Milner, et al., 1998; Kandel, Schwartz, and Jessell, 2000; Schafe, Nader, Blair and LeDoux, 2001). Generally speaking, the process of learning and memory consolidation taking place during Pavlovian conditioning involves the transformation of recently acquired labile associations from short-term-memory (STM) into long-term-memory (LTM) that is longer lasting and more stable (Davis and Squire, 1984; Schafe, et al., 2001). For example, during Pavlovian fear conditioning the pairing of a neutral stimulus (i.e. light or tone) with footshock the UCS initially results in the development of a transient short-term fear memory phase, but with repeated training and the passage of time, this short-term fear memory phase is efficiently transformed into a long-term fear memory phase that is both stable and enduring and easily accessed by simple conditioned stimuli (e.g. light or tone) that act as mnemonic devices (Davis and Squire, 1984; also see Schafe, et al., 2001; Kandel, 2000). In this form the memories associated with the CS or other fear eliciting stimuli (e.g. sound and smell of a previously encountered predator) are biologically adaptive because in a decisive moment they can quickly be retrieved in order to warn an organism of danger and help it to initiate autonomic and behavioural responses that lead to escape and increased chances of survival.

6.13: LTP, Like Memory Comes in Phases: Short-Term-Potentiation and Early LTP

As mentioned above, LTP also consists of distinct temporal phases and although LTP has been described as having several mini-phases such as short-term-potentiation (STP) as well as LTP1, LTP2, and LTP3, it is generally accepted that LTP is made up of two major phases (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). The initial phase is called early LTP (E-LTP) and can easily be dissociated from the second phase called late LTP (L-LTP). For example, both STP (the

precursor to E-LTP) and E-LTP can be easily distinguished from the L-LTP (i.e. LTP2 and LTP3) since STP and E-LTP has a faster rate of decay than L-LTP (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, 2000). Specifically, STP that is produced by a single high frequency train of stimulation generally decays in a few minutes after its induction and often lasts less than one hour (Bliss and Collingridge, 1993). Furthermore, STP generated in the hippocampus and lasting a few minutes in duration does not seem to depend on either protein or messenger RNA synthesis as protein inhibitors that normally suppress calcium phospholipids-dependent protein kinase (PKC) and block LTP induction seem to have limited impact on STP (Lovinger, Wong, Murakami and Routtenberg, 1987; Reymann, Frey, Jork, and Matthies, 1988; Malinow, Madison and Tsien, 1988; Malenka, 1989) However, Bliss and Collingridge (1993) point out that high doses of PKC enzyme inhibitors have been shown to block STP indicating that STP and perhaps very early forms of LTP may rely on simple modifications to existing proteins or calcium sensitive enzymes whose task is to initiate the sequence of biochemical events that convert the signals induced by calcium entry into the cell via the NMDA channel into calcium-mediated events that convert STP into E-LTP and the more stable L-LTP (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, et al., 2000). In a similar fashion E-LTP lasting anywhere from a few minutes to up to three hours can be induced in the hippocampus and the lateral nucleus of the amygdala by a single train of high frequency stimulation and as in the case of STP, research indicates that early LTP is also not dependent on either protein or messenger RNA synthesis (Huang, et al., 1994; Nguyen and Kandel, 1996; Nguyen et al., 1994; Huang, et al., 2000; Huang and Kandel, 1998). Also, while E-LTP is somewhat more resilient than STP, it generally will not survive beyond the three hour mark unless it is converted into the more stable form of LTP called L-LTP (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, et al., 2000).

6.14: Late Phase LTP

Late LTP which likely encompasses the mini-phases of LTP2 and LTP3 described by Bliss and Collingridge (1993) is generally much longer lasting than either STP or early LTP (Schafe, et al., 2001; Kandel et al., 2000). In most instances, L-LTP can last for several hours (i.e. 3 to 6 hours) as in the case of late LTP1 and LTP2 or it can last for periods that

range from 24 hours to several days as in the case of LTP3 (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, et al., 2000). Late phase LTP (i.e. primarily LTP2 and LTP3) is induced in the hippocampus and the lateral amygdaloid nucleus by the presentation of numerous high frequency tetanizing stimulations applied to the major sensory afferents that target these two brain regions and there is a strong indication that the late phase of LTP relies on protein and messenger RNA synthesis (Huang, et al., 2000; Nguyen and Kandel, 1996; Nguyen, et al., 1994; Frey, et al., 1993; Bliss and Collingridge, 1993; Kandel, et al., 2000). Furthermore, L-LTP is believed to be the crucial phase during which structural and biochemical modifications to synapses begin to take place (Kandel, 1997; Milner, et al., 1998; Schafe, et al., 2001; Kandel, et al., 2000) and it is at the level of the synapse where presynaptic and postsynaptic modifications may combine to influence cellular activity and form the molecular basis of learning and memory (i.e. the engram). The obvious questions that need to be answered are; what are the specific receptor subtypes and biochemical events that are involved in LTP, how does E-LTP lead to L-LTP and how are these events related to fear learning and fear memory consolidation? In other words, what research evidence gathered from animal studies into fear learning and memory consolidation supports the supposition that biochemical events associated with LTP in the amygdala are tantamount to a molecular form of memory? In order to accomplish this task, it is necessary to briefly discuss the sequence of biochemical and molecular events that are typically associated with early and late LTP in the hippocampus and the amygdala and then integrate this information with research studies that have experimentally manipulated the amygdala during fear conditioning.

6.15: Biochemical and Molecular Events Associated with LTP and Their Impact on Conditioned Fear Learning, Fear Memory Consolidation and Retrieval, and Fear Expression

Over the past decade or so many of the biochemical and molecular cascades associated with LTP induction in the hippocampus and lateral and basolateral amygdala have been uncovered and described in considerable detail (Kandel, 1997; Milner, et al., 1998; Malenka and Nicoll, 1998; Huang, Martin and Kandel, 2000; Huang and Kandel, 1998; Schafe, Atkins, Swank, Bauer, Sweatt and LeDoux, 2000; Brambilla, Gnesutta,

Minichiella, White, Roylance, Herron, Ramsey, Wolfer, Cestari, Arnaud, et al., 1997; Weisskopf, Bauer, and LeDoux, 1999; Maren, 1999; Nguyen and Kandel; 1996; Chapman, et al., 1990; Bliss and Collingridge, 1993; Kandel, et al., 2000; Rodrigues, Bauer, Farb, Schafe, and LeDoux, 2002). Generally speaking, much of this research suggests that LTP induction in both the hippocampus and the amygdala (i.e. the lateral and basolateral nuclei) is likely initiated by the entry of calcium into certain postsynaptic neurons after tetanic stimulation is applied to afferent fibres which innervate these two limbic regions (Chapman, et al., 1990; Bliss and Collingridge, 1993; Kandel, et al., 2000; Nguyen and Kandel, 1996; Huang and Kandel, 1998). Calcium entry into postsynaptic neurons is attributed to the functional activation of the NMDA receptor channel (Kandel, et al., 2000) and is triggered by L-glutamate binding to the NMDA receptor complex and fast acting postsynaptic membrane depolarizations instigated by non-NMDA receptors (i.e. AMPA) that become active during periods of high frequency tetanic stimulation (Kandel, et al., 2000). Hence, the binding of L-glutamate in combination with the excitatory postsynaptic potentials (EPSPs) produced by AMPA receptors depolarizing the postsynaptic membrane serve to activate the NMDA receptor channels and make them significantly more permeable to calcium (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, et al., 2000). The NMDA receptor channel normally contains a magnesium cation (Mg^{2+}) that acts as a voltage-dependent switch that occupies a site near the channel pore opening and in effect behaves much like a loosely fitting cork that plugs the opening of a wine bottle (Kandel and Siegelbaum, 2000; in Kandel, Schwartz and Jessell, 2000). When the NMDA receptor is activated the Mg^{2+} block is removed to allow calcium to enter the cell.

A second way in which calcium can enter the postsynaptic neuron is through the voltage-gated calcium channels (VGCCs) that become activated and open in response to the depolarization of the membrane near the channel pore (Siegelbaum and Koester, 2000 in Kandel, Schwartz and Jessell, 2000). Energy created from the change in the membrane potential (i.e. depolarization) triggers conformational changes in the receptor channel near the ion pore which allow it to open and permit calcium entry into the intracellular region (Siegelbaum and Koester, 2000 in Kandel, Schwartz and Jessell, 2000). Once calcium ions enter the intracellular domain, some may bind to a recognition site on the receptor complex to inactivate the channel and close the ion pore, whilst other calcium ions may be left free

to travel in the intracellular region where they can initiate further biochemical cascades and cellular events that eventually lead to synaptic modifications that are indicative of LTP (Siegelbaum and Koester, 2000; Kandel, 2000; Kandel and Siegelbaum, 2000 in Kandel, Schwartz and Jessell, 2000). Hence, calcium is believed to enter the postsynaptic cellular domain at a point located near the end of the dendritic spine which contains numerous NMDA and AMPA receptors and research indicates that calcium enters either via the NMDA receptor or via the L-type voltage-gated calcium channel.

As mentioned above, the NMDA receptor complex contains the magnesium cation that acts as a voltage-regulated gate that governs the flow of calcium into the postsynaptic cell and like the voltage-gated calcium channel, the NMDA receptor also requires sufficient cellular depolarization to become active (Kandel, et al., 2000; Collingridge, Kehl, and McLennan, 1983; Malenka and Nicoll, 1993; Wieskopf, Bauer and LeDoux, 1999; Grover and Teyler, 1990; Huang and Malenka, 1993; Magee and Johnston, 1997; Kandel and Siegelbaum, 2000 in Kandel, Schwartz and Jessell, 2000). In essence, at least two basic prerequisites must be met and occur in a simultaneous fashion before the NMDA channel opens to allow calcium to enter the postsynaptic cell. First, the membrane of the postsynaptic cell must be sufficiently depolarized in order to trigger the expulsion of the magnesium cation that normally blocks the ion channel of the NMDA receptor (Bliss and Collingridge, 1993; Kandel, et al., 2000). This depolarization process can be triggered either by high frequency tetanic stimulation or possibly by exposure to aversive environmental stimuli (e.g. footshock during Pavlovian fear conditioning) that produce fast AMPA-mediated EPSPs that eventually activate the NMDA receptor complex, voltage-dependent calcium channels or both. Second, L-glutamate must be present and bound to the NMDA receptor complex at the time of depolarization so as to promote the opening of the channel pore (Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, et al., 2000 in Kandel, Schwartz and Jessell, 2000). Indeed the special biochemical requirements along with the restrictions on calcium influx imposed by the magnesium cation found on the voltage-gated NMDA receptor complex allow it to operate as a molecular coincidence detector that helps with the formation and storage of biologically relevant memories that are acquired either during classical conditioning or through experience (Bliss and Collingridge, 1993; Li, Phillips and LeDoux, 1995; Maren, 1999; Schafe, et al., 2001;

Kandel, et al., 2000). Hence, it can be stated that the simultaneous activity of numerous afferents operating in conjunction with the pre to postsynaptic neuronal depolarization transfers and the activation of the NMDA receptor complex that enhances calcium entry into the postsynaptic cell generally follows the principals of cooperativity and associativity established by Hebb (1949) and various pioneering scientists who have studied LTP (Bliss and Lómo, 1973; Collingridge, et al., 1983; Lynch and Baudry, 1984; Manilow, et al., 1989; Grover and Teyler, 1990; Bliss and Collingridge, 1993; Frey, et al., 1993; Huang and Malenka, 1993; Malenka and Nicoll, 1993; Huang and Kandel, 1994; Nguyen, et al., 1994; Huang and Kandel, 1998; Mahanty and Sah, 1998; Malenka and Nicoll, 1999; Weisskopf, et al., 1999; Kandel, 2000; Kandel, et al., 2000 in Kandel, Schwartz and Jessell, 2000).

Following its entry into the postsynaptic cell, calcium (Ca^{2+}) is thought to facilitate early LTP induction by activating numerous Ca^{2+} sensitive enzymes (e.g. calpain, calcineurin, and certain phospholipases) and protein kinases (Oliver, Baudry and Lynch, 1989; Halpain, and Greengard, 1990; Schafe, et al., 2001; Bliss and Collingridge, 1993; Kandel, et al., 2000). At present, it is believed that Ca^{2+} influx into the postsynaptic cell promotes early modifications in synaptic efficiency (i.e. E-LTP) by activating two key protein kinases, these include; α-Ca^{2+}/calmodulin-dependent kinase II (αCaMKII) and the Ca^{2+}/phospholipids-dependent protein kinase (PKC), however a couple of other protein kinases such as cyclic-adenosine monophosphate-dependent protein kinase (i.e. PKA) and the tyrosine protein kinase Fyn can be activated as well (Kandel, 2000; Schafe, et al., 2001; Huang and Kandel, 1994; Malinow, Schulman and Tsien, 1989). Upon activation, αCaMKII and PKC can be autophosphorlated or continuously active for a long time after tetanic stimulation has been terminated and these kinase molecules can remain active even in the absence of Ca^{2+} (Soderling and Derkach, 2000; Sweatt, et al., 1998). During this persistently active phase αCaMKII and PKC have the ability to phosphorylate certain target protein molecules that may aid in the formation of new AMPA receptors on the postsynaptic membrane and/or activate receptor channels that are usually silent (Schafe, et al., 2001; Kandel, 2000). A specific example of such activity would be the autophosphorylated αCaMKII on Thr^{286} which usually results in the formation of new non-NMDA (i.e. AMPA) receptors on the surface of the postsynaptic membrane at the level of the dendritic spine (Nayak, Zastrow, Licteig, Zahniser and Browning, 1998; Soderling and

Derkach, 2000; Kandel, 2000; Schafe, et al., 2001). In a related fashion, αCaMKII also has the capacity to dramatically increase the sensitivity of non-NMDA receptors to glutamate, thus reducing the amount of glutamate required to induce postsynaptic depolarizations (Kandel, 2000). The emergence of new receptor channels and the conversion of the many otherwise silent non-responsive receptor channels into active receptors via αCaMKII-mediated actions, undoubtedly have a profound impact on the functioning of the postsynaptic cell as these changes may not only enhance and/or amplify excitatory signals that are directed into the postsynaptic neuron but may also may lead to further synaptic efficacy in the long-term (Nayak, et al., 1998; Soderling and Derkach, 2000; Kandel, 2000; Schafe, et al., 2001).

6.16: Differentiating the E-LTP Phase from the L-LTP Phase

At this point, it is necessary to make a clear distinction between E-LTP and L-LTP. Basically, E-LTP in the amygdala and hippocampus can be induced by a single tetanic stimulation, and it has several characteristic features that make it distinct from L-LTP (Huang, et al, 1994; Nguyen and Kandel, 1996; Huang, Martin, and Kandel, 2000; Huang and Kandel, 1998; also see Schafe, et al., 2001; Kandel, 2000). These features include, the commencement of shorter term Ca^{2+}-mediated events, simple modifications to existing proteins, and increases in the probability of transmitter release, although both phases of LTP do involve the activation of NMDA receptors and voltage-gated calcium channels (Bliss and Collingridge, 1993; Kandel, 2000; in Kandel, Schwartz and Jessell, 2000; Nguyen and Kandel, 1996; Huang and Kandel, 1998; Schafe, et al., 2001; Bauer, Schafe and LeDoux, 2002; Weisskopf, Bauer, and LeDoux, 1999). Furthermore, these E-LTP events do not rely on new protein or messenger ribonucleic acid (mRNA) synthesis nor do they generally result in any structural modifications (Schafe, et al., 2001; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). Basically, this means that E-LTP triggered by a single tetanic stimulation or a single Pavlovian conditioning trial may not proceed to the point where newly formed proteins or mRNA could act to promote stable and long-term changes in synapses. Nevertheless, E-LTP is important in that it can be considered to be a catalyst or a biochemical stepping stone that is necessary for L-LTP to become firmly established. In this sense, there is probably some degree of overlap between the two phases

of LTP with regards to the sequencing of certain early biochemical events such as NMDA receptor activation, Ca^{2+} entry into the postsynaptic cell and the activation of PKC and αCaMKII (two calcium-dependent serine-threonine protein kinases) (Kandel, 2000).

In contrast to E-LTP, the more developed phase of LTP (i.e. L-LTP) is the product of multiple high frequency trains of stimulation (Kandel, 2000; Schafe, et al., 2001). L-LTP like E-LTP relies upon the entry of Ca^{2+} into the postsynaptic membrane via the NMDA receptor complex or voltage-dependent calcium channels (Collingridge, Kehl and McLennan, 1983; Malenka and Nicoll; 1993; Bliss and Collingridge, 1993; Reymann, et al., 1989; Grover and Teyler, 1990; Huang and Malenka 1993; Weisskopf, Bauer and LeDoux, 1999; Bauer, Schafe and LeDoux, 2002). However, both NMDA receptors and voltage-dependent calcium channels (VDCCs) may be involved in facilitating Ca^{2+} entry into the postsynaptic cell but this depends to a large degree on tetanus stimulus parameters such as frequency, intensity, and duration of electrical stimulation used during artificial LTP induction (Grover and Teyler, 1990; Magee and Johnston, 1997; Cavus and Teyler, 1996; Morgan and Teyler, 1999). For example, LTP induction techniques that employ either high frequency tetanizing stimulation (i.e. greater than 200 Hertz) or the pairing of a weak presynaptic stimulation with a strong spike eliciting postsynaptic depolarization (i.e. coupling of pre and post synaptic inputs) usually activates voltage-gated calcium channels (Grover and Tyler, 1990; Magee and Johnston, 1997). In marked contrast, lower frequency tetanizing stimulation around the 30 Hertz mark causes prolonged cellular depolarization and recruits NMDA receptors for LTP induction (Cavus and Teyler, 1996). Finally, mid-intensity tetanus stimulation at frequencies between 100 to 200 Hertz has been shown to encourage the involvement of both NMDA receptors and voltage-gated calcium channels in promoting the influx of Ca^{2+} into the postsynaptic cell and in generating LTP (Cavus and Teyler, 1996; Morgan and Teyler, 1999). In any event, what is important to stress is that Ca^{2+} may enter the postsynaptic cellular domain directly via the ionotropic NMDA magnesium voltage-gated receptor channel, or via voltage-dependent calcium channels or indirectly by way of metabotropic glutamate receptors (mGluRs) that rely on second messenger systems which utilise G-proteins to activate adenylyl cyclase and phospholipase C (PLC) (Huang and Kandel, 1998; Weisskopf, et al., 1999; Nguyen and Kandel, 1996; Siegelbaum, Schwartz and Kandel, 2000; in Kandel, Schwartz and Jessell, 2000).

Two of the most prominent mGluRs involved in mediating LTP and memory formation are mGluR1 and mGluR5 subtypes which belong to the family of Group I metabotropic glutamate receptors (Balazs, Miller, Romano, de Vries, Chun, and Cotman, 1997; Huber, Sawtell, and Bear, 1998; Rodrigues, Bauer, Farb, Schafe and LeDoux, 2002; Siegelbaum, Schwartz and Kandel, 2000; in Kandel, Schwartz and Jessell, 2000). Metabotropic glutamate receptors can increase intracellular Ca^{2+} levels in the postsynaptic neuron by either recruiting Ca^{2+} ions stored in the endoplasmic reticulum, as is the case with the phosphoinositol system where inositol 1, 4, 5- triphosphate (IP_3) binds to a receptor located on the endoplasmic reticulum to release internal Ca^{2+} stores, or by acting directly on ion channels by activating a G-protein or second messenger (Siegelbaum, Schwartz and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). Thus, mGluRs can increase intracellular Ca^{2+} levels in the postsynaptic neuron in two ways. First, mGluRs can elevate intracellular Ca^{2+} by recruiting Ca^{2+} ions stored in the endoplasmic reticulum of the postsynaptic cell. This is accomplished in the phosphionositol system by IP_3 that is initially activated by PLC. Upon activation, the IP_3 molecule binds to a receptor site located on the endoplasmic reticulum causing the receptor's ion pore to open and release the internal Ca^{2+} stores (Siegelbaum, Schwartz and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000).

The second way mGluRs can increase intracellular Ca^{2+} levels is by acting directly on ion channels through a G-protein or second messenger. In the case of the cyclic AMP system, the G-protein activates adenylyl cyclase which in turn activates the second messenger cyclic AMP. Cyclic AMP then activates cAMP-dependent protein kinase which then leads to the phosphorylation of proteins that may contribute to biochemical processes that lead either to second messenger induced ion channel regulation or to the production of new receptors that help amplify and distribute synaptic signals more efficiently (Siegelbaum, Schwartz and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). Once this has occurred, Ca^{2+} ions may be allowed to enter into the intracellular domain from the extracelluar matrix in much greater quantities. This type of increased Ca^{2+} influx could, under the right circumstances, help strengthen LTP during fear conditioning. Hence the down-stream effects triggered by mGluRs that employ G-proteins and second messengers can have a profound impact on LTP induction and maintenance especially since the brain

contains a vast quantity of G-proteins (Siegelbaum, Schwartz and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000).

With regards to the ionotropic glutamate receptors, Ca^{2+} entry triggers a similar series of biochemical events to those in E-LTP that have been described earlier. The key difference between the two LTP phases is that L-LTP requires the synthesis of new mRNA and proteins (Schafe, et al., 2001; Kandel, 2000). Also L-LTP is reliant upon the cyclic-AMP dependent protein kinase (PKA) as well as the mitogen-activated protein kinase (MAPK) that together form the key components in the cAMP-PKA-MAPK-CREB signalling pathway that is involved in LTP induction and maintenance and associative learning and fear memory consolidation (Wu, Lu, Chang and Gean, 1999; Kandel, 2000; Schafe, et al., 2001; Bliss and Collingridge, 1993; Atkins, Selcher, Petraitis, Trzaskos and Sweatt, 1998; Schafe and LeDoux, 2000; Josselyn, Shi, Carlezon, Neve, Nestler and Davis, 2001; Stork and Pape, 2002). During L-LTP numerous high frequency trains lead to activation of NMDA receptors, second messengers, and Ca^{2+} influx into the postsynaptic neuron. With repeated stimulation αCaMKII and PKC are encouraged to recruit adenylyl cyclase which in turn activates the cyclic-AMP (adenosine monophosphate) and the cyclic-AMP dependent protein kinase (PKA) as well as MAPK. These protein kinase molecules are then free to migrate (i.e. translocate) to the nucleus where they act to phosphorylate the cAMP response-element binding protein (CREB) which is an important activator of gene transcription (Alberini, et al., 1995; Bacskai, Hochner, Mahaut-Smith, Adams, Kaang, Kandel, and Tsien,1993; Martin, Michael, Rose, Barad, Casadio, Zhu, and Kandel, 1997; Schafe, et al., 2001; Kandel, 2000; Impey ,Obrietan, Wong, Poser, Yano, Wayman, Deloulme, Chan, and Storm, 1998; Stork and Pape, 2002). Upon activation, CREB interacts with various DNA related mechanisms localized in the cell nucleus and this process leads to the transcription of the cAMP response element (CRE), the synthesis of new mRNA, and the eventual assembly of new proteins that are responsible for instigating long-term structural and biochemical changes to synapses, dendritic spines, and postsynaptic receptors (Kandel, 1997; Milner, et al., 1998, Malenka and Nicoll, 1999; Silva, Kogan, Frankland, and Kida, 1998; Schafe, et al., 2001; Kandel, 2000; Impey, et al., 1998; Stork and Pape, 2002).

CREB-CRE-mediated actions working in concert with the newly synthesized proteins likely accomplishes this task and enhances synaptic efficiency by initiating modifications to both the presynaptic and postsynaptic cellular regions (Kandel, 2000; Bliss and Collingridge, 1993; Schafe, et al., 2001; Kandel, 1997, Milner, et al., 1998; Malenka and Nicoll, 1999; Silva, et al., 1998). This could involve changes such as the formation of new receptor channels (i.e. formation of new AMPA or NMDA receptors) on existing dendritic spines, increasing the number of dendritic spines with glutamatergic receptor channels, creating more neurotransmitter docking stations in the presynaptic cell, increasing the probability of neurotransmitter release, activating more G-proteins and second messenger-mediated events and making glutamatergic receptor channels more sensitive to L-glutamate and depolarizing currents that are produced by various forms of sensory stimuli (e.g. light + shock trials during fear conditioning) (Soderling and Derkach, 2000; Kandel, 2000; Schafe, et al., 2001; Bliss and Collingridge, 1993). This type of activity could provide the molecular and biochemical composition or structure onto which fear memories could be permanently stored (Kandel, 2000; Schafe, et al., 2001; Josselyn, et al., 2001; Maren, 1999; Sweatt, 1999). In other words, long-term synaptic modifications, cellular alterations, and biochemical cascades that occur in the amygdala may form the molecular basis of fear learning and fear memory formation (Schafe and LeDoux, 2000; Schafe, et al., 2001; Maren, 1999; Kandel, 2001; Rodrigues, et al., 2002; Stork and Pape, 2002).

6.2: Disrupting the cAMP-PKA-MAPK-CREB Signalling Pathway Impairs Conditioned Fear

In fact, research indicates that the administration of pharmacological agents into the lateral and basolateral amygdala, that disrupt the normal activities of the amygdaloidal cAMP-PKA-MAPK-CREB signalling pathway, are generally quite successful in preventing conditioned fear learning and fear-memory consolidation especially when these drugs are introduced prior to fear conditioning (Goosens, Holt and Maren, 2000; Schafe, Atkins, Swank, Bauer, Sweatt and LeDoux, 2000; Schafe and LeDoux, 2000; Schafe, Nadel , Sullivan, Harris and LeDoux, 1999; Josselyn, et al., 2001; Stork and Pape, 2002). For example, bilateral intra-basolateral amygdaloid infusion of 1-(5'-isoquinolinesulfonyl)-2-methylpiperazine (H7), a potent inhibitor of PKC and PKA, just prior to auditory fear

conditioning left short-term fear memories intact when rats were tested immediately after training but completely blocked long-term conditional fear as measured by defensive freezing behaviour when the same animals were assessed twenty-four hours later (Goosens, Holt, and Maren, 2000). Furthermore, a dose-response experiment conducted by Maren and associates (2000) revealed that infusion of H7 into the BLA prior to contextual fear conditioning dose-dependently blocked long-term contextually conditioned fear memories when rats were tested twenty-four hours later but had no significant effect on the acquisition or expression of short-term conditional fear memory when it was assessed shortly after the context + footshock pairings were made. More specifically, the deficits in long-term conditional fear memories (i.e. to both auditory and contextual cues) produced by H7 seems to be attributed to the cascade of biochemical events occurring in the BLA during fear learning and memory consolidation since H7 infusions made into the central nucleus of the amygdala of rats prior to fear training failed to block tone specific and contextual fear memories when these animals were measured for defensive freezing behaviour twenty-four hours later (Goosens, et., al., 2000).

In addition, research carried out by Schafe and colleagues (2000) demonstrated that the extracellular-signal regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade must be activated in the amygdala in order for long-term memories of the Pavlovian fear conditioning experience to become firmly established (Schafe, Atkins, Swank, Bauer, Sweatt and LeDoux, 2000). This elegant research revealed that interrupting ERK/MAPK events in the basolateral amygdaloid complex by infusing the protein kinase inhibitor U0126 (a specific inhibitor of MEK that functions as a regulator of ERK/MAPK activation) into this limbic region blocked the formation of long-term fear memories but left short-term fear memories intact (Schafe, et al., 2000; Favata, Horiuchi, Manos, Daulerio, Stradley , Feeser, Van Dyk, Pitts, Earl, Hobbs, Copeland, Magolda, Scherle, and Trzaskos, 1998). Specifically, rats infused with a 1.0 μg dose of U0126 thirty minutes prior to auditory fear conditioning failed to exhibit significant levels of conditioned defensive freezing behaviour during the fear retention test conducted 24 hours after the last tone + footshock trial had been administered (Schafe, et al., 2000).

On the other hand, rats administered the vehicle solution (i.e. DMSO) or the lower dose of U0126 (i.e. 0.1 μg) before fear training displayed more robust freezing behaviour in the

presence of the auditory CS during the retention test (Schafe, et al., 2000). However, it is important to point out that the low dose of U0126 did seem to cause some mild albeit non-significant impairment on fear memory consolidation as rats in this particular group did not display as much defensive freezing behaviour at the 24 hour test interval. Furthermore, U0126 infusion into the basolateral amygdaloid complex did not disrupt short-term fear memory since no significant between group differences in CS-induced freezing behaviour were apparent during retention testing conducted at the one or three hours mark after fear conditioning had been completed (Schafe, et al., 2000). However, significant differences in the level of fear elicited defensive freezing between the vehicle control rats and the 1.0 μg U0126 rats did emerge at the six hour testing interval and became even more pronounced during the final test interval administered 18 hours later (Schafe, et al., 2000). These findings suggest that long-term fear memory consolidation was not impaired in the vehicle treated rats, only slightly impaired in the rats administered the 0.1 μg dose of U0126 but completely abolished in the 1.0 μg U0126 treated rats (Schafe, et al., 2000). In addition to this finding, immunohistochemical and Western blot testing revealed that paired but not unpaired fear conditioning trials, and tone + shock but not tone-alone or shock-alone presentations activated ERK/MAPK events in the lateral nucleus of the amygdala (Schafe, et al., 2000).

Moreover, it is noteworthy that Goosens, et al., (2000) findings regarding the role of amygdaloidal PKA activity in memory consolidation are generally congruent with that of Koh, Thiele, and Berstein (2002) who have recently shown that inhibition of PKA activity in the BLA before taste aversion training blocked the long-term memory of conditioned taste aversion but left short-term memory unimpaired. In this particular study, infusion of the selective PKA inhibitor Rp-adenosine 3'5-cyclic monophosphothioate triethylamine (Rp-cAMPS) into the BLA caused a deficit in long-term memories associated with taste aversion (Koh, et al., 2002), a result that closely parallels the work of Schafe and LeDoux, 2000 who found similar effects on fear memory consolidation when PKA inhibitors are injected into the amygdala. Taken together, the behavioural, pharmacological and immunohistochemical data discussed above provide convincing evidence that PKA and MAPK activation in the BLA is required for long-term fear memory consolidation.

With reference to studies that employ FPS as a method to examine conditional fear, research has also shown that protein kinase inhibitors and/or drug manipulations that interfere with either MAPK or the transcription factor CREB also block the formation of long-term memory in fear conditioning (Josselyn, et al., 2001; Lin, Yeh, Lin, Lu, Leu, Chang, Gean, 2001). For example, the phosphatidylinositol 3-kinase (PI-3 kinase) has been shown to become activated in the amygdala but not in the hippocampus and cerebellum in rats that have received Pavlovian fear conditioning (Lin, et al., 2001). Activation of PI-3 kinase was shown to occur following LTP induction in the BLA that had been generated by tentanic stimulation of the external capsule (Lin, et al., 2001). In addition to this, the selective kinase inhibitors wortmannin and LY 294002 blocked amygdaloid LTP induction, amygdala-based PI-3 kinase activation, and the tetanus and forskolin-induced activation of MAPK (Lin, et al., 2001). Furthermore, CREB activation triggered by fear conditioning, tetanic stimulation, or forskolin application to amygdala slices could be blocked by pre-treatment with these two protein kinase inhibitors (Lin, et al., 2001). More importantly, Lin and colleagues (2001) demonstrated that bilateral microinfusions of wortmannin into the basolateral amygdaloid complex of rats 30 minutes prior to fear training resulted in a dose-dependent blockade of FPS when these animals were tested for the retention of this fear memory 24 hours later. In another similarly related experiment, these researchers found that rats treated with intra-amygdala infusion of wortmannin (5.0 μg dose) before fear conditioning commenced only exhibited FPS one hour after fear training but not when they were tested 24 hours later, which seemed to indicate that long-term but not short-term fear memories for the CS were impaired (Lin, et al., 2001). Taken together, these results seem to indicate that fear conditioning and LTP induction trigger a similar sequence of biomolecular events in the amygdaloidal cAMP-PKA-MAPK-CREB signalling pathway and the inhibition of protein kinase activity in the amygdala seems to disrupt both LTP and long-term fear memories. Consistent with this notion is the research that indicates that CREB activation and syntheses of mRNA is required for the acquisition and formation of long-term fear memories (Josselyn, et al., 2001; Bailey, Sun, Thompson, Kim, and Helmstetter, 1999).

It has been postulated that CREB activity that occurs within certain basolateral amygdaloid neurons during or shortly after fear conditioning can function like a

biomolecular switch that is capable of initiating cellular events such as the formation of new proteins and mRNA that serve to facilitate fear learning and the formation of long-term memory in fear conditioning (Josselyn, et al., 2001; Bailey, et al., 1999). Indeed, numerous research efforts have demonstrated that CREB is highly involved in regulating the production of proteins that are deemed necessary for the formation and/or consolidation of long-term memories in several different organisms including the marine snail Aplysia and rodents such as rats and mice (Dash, Hochner, and Kandel, 1990; Kaang, Kandel, and Grant, 1993; Impey, Smith, Obreitan, Donahue, Wade and Storm, 1998a; Bourtchouladze, Abel, Berman, Gordon, Lapidus, Kandel, 1998; Guzowski and McGaugh, 1997; Lamprecht, Hazui, and Dudia, 1997; Josselyn, et al., 2001; Bailey, et al., 1999). For example, increasing CREB levels in the basolateral nucleus of the amygdala of rats by employing a technique called herpes simplex virus vector-mediated CREB transfer (HSVm-CREBt) was shown to significantly enhance the weak long-term fear memories (i.e. increase FPS) that are normally produced by the presentation of massed fear conditioning trials that have short inter-trial intervals (Josselyn, et al., 2001). More specifically, increasing CREB activity in the BLA via HSVm-CREBt pre-treatment administered three days before massed fear conditioning significantly enhanced FPS responding in HSV-CREB-treated rats tested either 48 hours or 14 days after fear training. In contrast, vehicle control rats that were exposed to massed fear conditioning trials exhibited no enhancement of long-term fear memories when tested for potentiated startle at these time intervals (Josselyn, et al., 2001). The fear memory enhancement produced by CREB activation seems to be an effect mediated by BLA neurons as similar experimental manipulations carried out on the caudate-putamen failed to result in any increase in FPS responding (Josselyn, et al., 2001). In addition, CREB over expression in the BLA did not seem to facilitate a short-term memory for fear conditioning as rats treated using the HSVm-CREBt method did not display any significant changes in the level of short-term-memory when assessed for FPS 15 minutes after massed fear training (Josselyn, et al., 2001). This result obtained by Josselyn and colleagues (2001) is generally harmonious with the earlier experimental work of Lamprecht and associates (1997) who demonstrated that CREB binding in the amygdala is essential for the consolidation of long-term but not short-term conditioned taste aversion memory.

More recently, research conducted on a strain of genetically altered mice (i.e. CaMKIV$^{-/-}$ mice) that lack a key component of the calcium-calmodulin-dependent protein kinase that normally activates CREB found that these particular animals exhibited significant deficits in both auditory and contextual freezing behaviour when assessed either at one or seven days after Pavlovian fear conditioning (Wei, Qui, Liauw, Robinson, Ho, Chatila, and Zhou, 2002). In addition, immunoreactive experiments conducted by Wei, et al., (2002) demonstrated that the presentation of tone + footshock conditioning trials activated levels of phosphorylated CREB (i.e. pCREB) in the basolateral amygdaloid nucleus of wild mice. In stark contrast, pCREB activation in the BLA of CaMKIV$^{-/-}$ mice was significantly reduced or completely absent indicating that Ca^{2+}MKIV activity is essential for fear conditioning and is required to trigger CREB activation during aversive learning (Wei, et al., 2002). Moreover, BLA slice preparations obtained from CaMKIV$^{-/-}$ mice failed to exhibit any meaningful synaptic potentiation when exposed to theta-burst stimulation, whereas identical manipulations performed on BLA slices taken from genetically unaltered wild mice resulted in robust increases in synaptic potentiation (Wei, et al., 2002).

Thus, in addition to Ca^{2+}MKII, Ca^{2+}MKIV may also be an important calcium-dependent protein kinase that activates CREB and helps to facilitate LTP, fear acquisition, and the consolidation of long-term CS-UCS associations (Wei, et al., 2002). It has been suggested that Ca^{2+}MKIV accomplishes this task at the level of the cell nucleus since this particular protein kinase molecule is found in abundance in the nuclear region of neurons where it is has been shown to play a role in the regulation of CREB-induced gene expression that helps to initiate the synthesis of new proteins and mRNA that may play a fundamental role in facilitating synaptic modifications that enhance synaptic efficiency (Wei, et al., 2002; Deisseroth, Heist and Tsien, 1998; Ho, Liauw, Blaeser, Wei, Hanissian, Muglia, Wozniak, Nardi, Arvin, Holtzman, Linden, Zhou, Muglia, and Chatila, 2000).

6.21: Messenger RNA (mRNA), LTP and Conditioned Fear Memory

In addition to the involvement of amygdaloidal CREB activation in the development of long-term fear memories, research also indicates that new mRNA and the subsequent production of new proteins is important for the induction of neural plasticity and the long-term establishment of fear memories (Nguyen, Abel, and Kandel, 1994; Bailey, et al.,

1999). For example, pre-training bilateral infusions of the mRNA synthesis inhibitor actinomycin-D (act-D) into the basolateral nucleus of the amygdala in rats sharply attenuated conditioned defensive freezing behaviour to both auditory (i.e. 72 dB tone) and contextual cues that had been paired with foot shocks when rats were tested 25 hours after act-D infusion and fear conditioning (Bailey, et al., 1999). It is important to note that these results could not be attributed to damage produced by either the infusion process or the drug since the same rats that were administered act-D and displayed fear memory deficits all showed normal learning and fear expression when they were retrained and then tested for defensive freezing one week later after the major experiment had been completed (Bailey, et al., 1999).

In a similar vein, studies exploring the role of protein synthesis inhibitors on fear memory consolidation as measured by freezing behaviour demonstrated that infusion of the protein synthesis inhibitor anisomycin into the basolateral amygdaloid complex shortly after Pavlovian fear conditioning completely blocked defensive freezing behaviour to an auditory CS suggesting that anisomycin prevented the establishment of enduring CS-UCS associations (Schafe and LeDoux, 2000). In this particular study, the infusions of anisomycin blocked long-term fear memories but had no impact on short-term fear memories as rats tested shortly after anisomycin infusion exhibited defensive freezing behaviour but failed to display this behavioural response when tested the following day (Schafe and LeDoux, 2000). Furthermore, Nader, Schafe and LeDoux, (2000) showed that anisomycin microinfusions made into the BLA immediately after the reactivation of a fear memory, induced via a short behavioural test, produced amnesia for the original CS-UCS learning experience when the rats were retested for conditioned defensive freezing behaviour 24 hour later. However, when intra-amygdalar anisomycin infusions were made 6 hours after reactivation (i.e. retrieval) of the original fear memory this particular protein synthesis inhibitor had no effect on conditioned defensive freezing measured 24 hours later (Nader, et al., 2000). This seems to indicate that reactivation of old fear memories temporarily places these CS-UCS fear memories into an unstable state that may then require reactivation of protein synthesis to refresh and keep such memories stable so that they are useful to the organism in future situations (Nader, et al., 2000). These results are generally consistent with previous work that demonstrated the importance of PKA and

MAP kinases in fear memory consolidation when either auditory or contextual conditioned stimuli were used during fear training and testing (Schafe, et al., 1999).

6.3: The Role of Glutamate Receptors in Mediating LTP, Fear Learning, and Synaptic Transmission in the Amygdala

Although many of the biochemical events and molecular mechanisms associated with LTP, fear learning, and fear memory storage are undoubtedly complex, numerous research studies and research reports seem to indicate that they initially involve the activity of NMDA and/or AMPA/kainate glutamate receptors (Miserendino, Sananes, Melia and Davis, 1990; Campeau, Miserendino and Davis, 1992; Kim, Campeau, Falls, and Davis, 1993; Fanselow and Kim, 1994; Liang, Hon, and Davis, 1994; Maren and Fanselow, 1995; Li, Phillips and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997; Huang and Kandel, 1998; Maren, 1999; Walker and Davis, 2000; Walker and Davis, 2002; Fendt, 2001; Goosens and Maren, 2003). Like the hippocampus, the lateral and basolateral nuclei of the amygdala also contain high concentrations of excitatory amino acid receptors (Monaghan, and Cotman, 1985; Farb and LeDoux, 1994; McDonald, 1994; Farb, Aoki, and LeDoux, 1995; Weisskopf and LeDoux, 1999). These include NMDA receptors (Monaghan and Cotman, 1985) located on postsynaptic dendritic spines (Malenka, Kauer, Perkel, and Nicoll, 1989) where most thalamo-amygdala fibres have been shown to terminate (Farb, LeDoux, and Milner, 1989). A number of studies have demonstrated that LTP can be induced in the lateral or basolateral nucleus of the amygdala (Chapman and Brown, 1988; Chapman, Kairiss, Keenan and Brown, 1990; Maren and Fanselow, 1995; Rogan and LeDoux, 1995; Huang and Kandel, 1998; Weisskopf, et al., 1999; Huang, Martin and Kandel, 2000) and this can be accomplished by high frequency stimulation of the external capsule (Chapman and Brown, 1988), hippocampus (Maren and Fanselow, 1995) auditory thalamus (Clugnet and LeDoux, 1990) and efferent cortical projections to the amygdala (Chapman, Kairiss, Keenan and Brown, 1990; Yaniv and Richter-Levine, 2000).

It is also very important to highlight the fact that LTP has been shown to exist in many of the key sensory pathways that project to the lateral and BLA and this research strongly suggests that the vast majority of these pathways that target neurons of the basolateral

amygdaloid complex are critical for fear learning and memory consolidation during Pavlovian fear conditioning as they provide auditory, somatosensory, visual and nociceptive information to the amygdala (Chapman, et al., 1990; Clugnet and LeDoux, 1990; Huang, et al., 2000; Weisskopf, et al., 1999; Rogan and LeDoux, 1995; Huang and Kandel, 1998; Maren and Fanselow, 1995; for a review of sensory cascades to the amygdala see McDonald, 1998; Ono, et al., 1995; Uwano, et al., 1995; Shi and Davis, 1999; also see Chapter 3 of this thesis). More interestingly, Pavlovian fear conditioning has been shown to produce a significant enhancement of cellular activity and functioning (i.e. LTP) at the point where sensory inputs terminate in the basolateral amygdaloid complex and this type of fear-induced neuronal activation is nearly identical to the kind of LTP produced by high frequency tetanizing electrical stimulation (McKernan and Shinnick-Gallagher, 1997; Rogan, Staubli and LeDoux, 1997a; Blair, Tinkelman, Moita and LeDoux, 2003).

Since LTP in the amygdala possibly relies on NMDA receptor, AMPA receptor, mGluR, or voltage-dependent calcium-channel-mediated-events and can be produced either by tetanic stimulation or by Pavlovian fear conditioning techniques, it is highly probable that synaptic modifications and long-term biochemical changes to amygdaloid neurons and pathways are vital for fear acquisition and fear memory consolidation (Chapman, et al., 1990; Clugnet and LeDoux, 1990; Rogan, et al., 1997a; McKernan and Shinnick-Gallagher, 1997). For instance, it has been shown that NMDA and non-NMDA receptors contribute to and facilitate synaptic transmission between the medial geniculate body and the lateral nucleus of the amygdala (Li, Phillips, and LeDoux, 1995; also see McKernan and Shinnick-Gallagher, 1997) and since the lateral and basolateral nuclei of the amygdala receive auditory information from the mdMGN and PIN of the thalamus (via the extralemniscal pathway) and visual information from the dorsal lateral geniculate and lateral posterior nuclei of the thalamus, it is likely that these pathways along with the LTP-like events occurring within them represent a circuit that is vital for fear learning and expression (LeDoux, et al., 1990; Romanski and LeDoux, 1993; Romanski, et al., 1993; Iversen, et al., 2000; Shi and Davis, 2001).

Research has shown that LTP events have also been generated in the amygdala by stimulation of the hippocampal-amygdalar pathway at the level of the ventral angular

bundle which emerges out of the subiculum and CA1 region of the hippocampus (Maren and Fanselow, 1995; Ottersen, 1982; Canteras and Swanson, 1992). This finding provides direct physiological evidence that helps clarify how contextual information processing in the hippocampus might interact with the emotional response centres of the amygdala at a cellular level to facilitate contextual fear conditioning (Phillips and LeDoux, 1992; Maren and Fanselow, 1995). Thus, hippocampal innervation of the amygdala and LTP occurring in this pathway may make up the neural circuitry that is involved in mediating contextual fear learning and memory consolidation (Phillips and LeDoux, 1992; LeDoux, 1993; Maren and Fanselow, 1995). This seems likely as neurons in the CA1 region of the hippocampal formation support contextual fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Kim, Rison and Fanselow, 1993; Maren and Fanselow, 1997; McNish, Gewirtz and Davis, 2000; Antoniadis and McDonald, 2000; Hall, Thomas and Everitt, 2001) and have been shown to innervate a number of amygdaloidal nuclear groups (i.e. accessory basal nucleus, lateral nucleus, basal nucleus, AHA, CLC, intercalated masses, BNST, CoA, and MeA) (see Canteras and Swanson, 1992; Ottersen, 1982; McDonald, 1998).

Pharmacological antagonism of NMDA receptors by the direct infusion of AP5 made into the lateral and basolateral nuclei has been shown to prevent fear learning (Miserendino, Sananes, Melia, and Davis, 1990; Fanselow and Kim, 1994; Walker and Davis, 2000) and fear expression (Lee and Kim, 1998; Fendt, 2001; Lee, Choi, Brown and Kim, 2001). This suggests that efficient amygdaloid NMDA receptor-mediated excitatory synaptic transmission is crucial for the successful learning and memory storage processes that occur during fear conditioning. With this in mind, it is important to point out that NMDA antagonists interfere with long-term but not short-term memory in spatial learning tasks (Kesner and Dakis, 1995; Steele and Morris, 1999) and avoidance tasks (Izquierdo, Izquierdo, Barros, Mello e Souza, de Souza, Quevedo, Rodriques, Sant'Anna, Madruga, and Medina, 1998) after infusions are made into the hippocampal formation of laboratory animals. Also, similar intra-hippocampal infusions of NMDA receptor blockers have been shown to prevent LTP induction in CA1 region of the hippocampus (Collingridge and Bliss, 1987). This raises the interesting possibility that similar NMDA-LTP-mediated synaptic processes observed in the hippocampus also occur in the amygdala during

Pavlovian fear conditioning trials and there are several electrophysiological and pharmacological experiments that support this supposition (Miserendino, et al., 1990; Campeau, Miserendino, and Davis, 1992; Fanselow and Kim, 1994; Li, et al., 1995; Rogan, et al., 1997a; McKernan and Shinnick-Gallagher, 1997; Gerwitz and Davis, 1997; Walker and Davis, 2000).

Electrophysiological recording studies have demonstrated that the dorsal subnucleus of the lateral amygdala contains a significant population of tone responsive neurons with short response latencies falling in the range of 10-25 milliseconds (Bordi and LeDoux, 1992; Bordi, et al., 1993; Romanski, et al., 1993; Quirk, Repa, and LeDoux, 1995; Quirk, Armony, and LeDoux, 1997). Many of these neurons in the dorsal subdivision of the lateral amygdaloid nucleus (LAd) have been shown to respond to auditory stimuli with shorter latencies and with a higher degree of consistency than neurons in the ventral subdivision of the lateral nucleus (Bordi and LeDoux, 1992; Bordi et al., 1993; Romanski et al., 1993; Quirk, et al., 1995). Simultaneous recordings obtained from multiple lateral amygdaloid neurons in freely moving rats during classical fear conditioning revealed that exposure to tone-shock pairings not only considerably increased the proportion of LAd cells that exhibited early tone responses but also altered the functional couplings between neurons in this region (Quirk, Repa, and LeDoux, 1995). In the same study, Quirk et al., (1995) also demonstrated that a small number of fear conditioning trials was sufficient to increase the magnitude of tone-elicited responses and to convert unresponsive neurons into tone responsive neurons. Taken together, the physiological and neurobiochemical properties of neurons in the lateral amygdaloid nucleus make it ideally suited as a site where auditory and somatosensory information can converge to support fear learning and LTP processes that are necessary for the consolidation of aversive emotional memories (Bordi, LeDoux, Clugnet, and Pavlides, 1993; Li, et al., 1995; Li, et al., 1995; McKernan and Shinnick-Gallagher, 1997; Quirk, et al., 1995; 1997; Rogan, et al., 1997a; Rogan and LeDoux, 1995).

Moreover, fear conditioning modifies evoked field potential responses in the lateral amygdaloid nucleus and thus produces CS-evoked potentials that are very similar in magnitude to the auditory and electrically-induced evoked potentials that are produced during LTP induction in anesthetized rats (Rogan, Stäubli and LeDoux, 1997a). In a related fashion, fear conditioning also enhanced the auditory tone evoked responses of

neurons in the lateral nucleus of the amygdala (Rogan, Stäubli and LeDoux, 1997a). These authors (Rogan, et al., 1997a) also reported a significant increase in the slope and amplitude of CS evoked potentials during fear conditioning and testing trials. It is important to note that these changes were only observed in rats that received paired conditioning trials, thus animals that received non-associative conditioning (i.e. explicitly unpaired CS-UCS trials) failed to demonstrate any significant increases in the slope and amplitude of CS evoked potentials nor did they display conditioned fear expression during the testing phase of the experiment (Rogan et al., 1997a). Thus, LeDoux and colleagues have demonstrated that the neural changes occurring within the lateral amygdala in response to fear conditioning are similar to those observed when LTP is physiologically induced (see Quirk, et al., 1995; 1997; Rogan and LeDoux, 1995; Rogan, et al., 1997a).

In related research, McKernan and Shinnick-Gallagher (1997) incorporated the FPS paradigm to study the electrophysiological and neurochemical properties of neurons obtained from the lateral amygdaloid nucleus of rats that received paired, unpaired or no Pavlovian fear conditioning training. This research revealed several findings that seem to support the view that LTP occurs in the amygdala and that glutamate receptors (i.e. NMDA, AMPA) may contribute to LTP, synaptic transmission, and fear learning and memory formation. First, electrophysiological research revealed that excitatory post-synpatic currents (EPSCs) in the lateral amygdala produced, by electrically stimulating fibres emanating from the medial geniculate nucleus of the thalamus, could be blocked by the co-application of the NMDA receptor antagonist AP5 and the AMPA receptor antagonist GYKI 52466. This finding seems to indicate that synaptic transmission and synaptic modifications may be mediated by NMDA and AMPA receptors in the thalamo-amygdalar pathway (McKernan and Shinnick-Gallagher, 1997). Second, EPSCs measured in lateral amygdala neurons obtained from rats exposed to paired fear conditioning trials were observed to be significantly enhanced, whereas EPSCs measured from the lateral amygdala neurons of naïve and unpaired control rats displayed no such potentiation (McKernan and Shinnick-Gallagher, 1997). More specifically, the slope of the input-output curve in lateral amygdaloid nucleus neurons obtained from paired fear conditioned rats was much larger than the slope from neurons of unpaired controls and the EPSC threshold was also reported to be significantly lower in fear conditioned rats versus the

naïve and unpaired control groups (McKernan and Shinnick-Gallagher, 1997). Also, when the AMPA-mediated component of the input-output curve was isolated in the presence of AP5 these researchers found that the slope of the input-output curve of fear conditioned animals was significantly larger than unpaired controls indicating that AMPA-mediated synaptic transmission is enhanced after Pavlovian fear conditioning (McKernan and Shinnick-Gallagher, 1997). This experimental work in conjunction with other research efforts (Bordi, et al., 1993; Romanski, et al., 1993; Quirk, et al., 1995; 1997; Rogan and LeDoux, 1995; Rogan, et al., 1997a; Rogan, et al., 1997b; Li, et al., 1995; Miserendino, et al., 1990; Walker and Davis, 2000; Rodrigues, Bauer, Farb, Schafe and LeDoux, 2002; Blair, Tinkelmann, Moita, and LeDoux, 2003) seems to indicate that strict Pavlovian fear conditioning leads to synaptic modifications in the lateral amygdala that likely involves glutamate receptor activation (i.e. NMDA, AMPA and metabotropic glutamate receptors).

As such, the amygdala may be a structure where information about the emotional significance of biologically relevant stimuli is permanently stored. In this way the amygdala through its various connections with other cortical and subcortical structures can add an emotional label to a memory or experience and thus direct the appropriate set of responses towards stimuli that have been associated with a positive or aversive event. This notion is supported by the fact that fear conditioning or exposure to a biologically relevant CS has consistently been shown to modify neural activity and tone evoked firing rates of neurons in a number of amygdaloid nuclei including the central nucleus (Pascoe and Kapp, 1985), the basolateral nucleus (Maren et al., 1991; Muramoto et al., 1993) and the dorsal and ventral subdivisions of the lateral nucleus of the amygdala (Ben-Ari and Le Gal La Salle, 1972; Segal, 1973; Quirk, et al., 1995; Quirk, et al., 1997; Rogan, et al., 1997a).

6.4: The Involvement of BLA NMDA Receptors in Conditioned Fear Learning, Memory Consolidation and Fear Expression

Pharmacological manipulations of the BLA have demonstrated that the acquisition of conditioned defensive freezing and FPS can be blocked when the NMDA antagonist AP5 is infused shortly before fear conditioning (Miserendino, Sananes, Melia and Davis, 1990; Campeau, Miserendino, and Davis, 1992; Fanselow and Kim, 1994; Lee and Kim, 1998; Walker and Davis, 2000; also see Walker and Davis, 2002). More specifically, infusion of

the competitive NMDA receptor antagonist AP5 into the BLA prior to fear conditioning dose-dependently blocked the acquisition of FPS in rats trained to a visual CS, whereas infusion with vehicle solution or propranolol had no effect (Miserendino, et al., 1990). In this particular study, Miserendino and colleagues (1990) demonstrated that microinfusion of AP5 (6.25, 12.5, 25, 50 nano-mole doses) into the BLA prior to a series of light + shock fear condition trials caused a dose-dependent blockade of FPS when animals were tested one week later. The effect of AP5 on conditional fear seems to be mediated specifically by NMDA receptors located on basolateral amygdaloid neurons since a 100 nano-mole AP5 infusion directed into the cerebellar interpositus nuclei of rats prior to fear training failed to block FPS responding (Miserendino, et al., 1990). The effects of NMDA receptors on fear learning do not seem to be restricted to one sensory modality because intra-amygdalar infusions of the NMDA receptor antagonist AP5 was also shown to block the acquisition of FPS in rats conditioned using an auditory CS (Campeau, Miserendino and Davis, 1992). However, this drug failed to block fear expression when it was infused just prior to fear testing in rats that had previously undergone fear training, suggesting that NMDA receptor antagonism in the amygdala does not affect fear expression (Campeau, et al., 1992). Moreover, second-order fear conditioning was significantly disrupted by antagonizing NMDA amygdaloid receptors (Gewirtz and Davis, 1997) indicating that NMDA receptors are involved in stimulus-stimulus learning.

In a similar manner, the acquisition of defensive freezing behaviour to a contextual CS was prevented by pretraining bilateral infusions of the NMDA antagonist D,L-2-amino-5-phosphonovalaric acid into the BLA (Fanselow and Kim, 1994). In addition, when the continuous multiple trial inhibitory avoidance tasks were employed to study fear motivated behaviour, pretraining intra-amygdalar infusions of NMDA receptor blockers (i.e. AP5, MK-801 and CPP) impaired inhibitory avoidance performance during retention testing, but failed to produce a blocking effect on inhibitory avoidance acquisition (Kim and McGaugh, 1992). Furthermore, intra-amygdala infusion of AP5 prior to post-testing does not attenuate the behavioural expression (i.e. inhibitory avoidance) of an already learned fear response (Kim and McGaugh, 1992; Liang, Hon, and Davis, 1994). This finding is consistent with Campeau and colleagues (1992) who found that AP5 infusion into the BLA before testing failed to block the expression of FPS. The only disparity between the

inhibitory avoidance results and those obtained from the FPS paradigm is that pretraining AP5 infusion into the amygdala does not block the acquisition of inhibitory avoidance (Kim and McGaugh, 1992).

In contrast, similar infusions block the initial formation of fear memories (i.e. acquisition) and the long-term fear memories (i.e. retention) in animals tested using the FPS paradigm (Walker and Davis, 2000). These differences may be explained by the fact that inhibitory avoidance acquisition involves a motor component and a conditioned fear memory/consolidation component (Hitchcock and Davis, 1986). Hence, it is possible that the NMDA receptor blockade by AP5 infusion disrupted the long-term fear memory/consolidation component but not the motor learning or the short-term memory component, thus making it possible for the animals to display inhibitory avoidance during the training phase but not during the retention phase which requires the long-term fear memory component. This explanation seems plausible since bilateral infusions of AP5 into the amygdala did not disrupt locomotor activity in an open field task (Liang, et al., 1994). Another possibility suggested by Walker and Davis (2000), is that FPS and inhibitory avoidance involves two different types of learning with each relying on specialized and independent neural substrates within the amygdala. For instance, inhibitory avoidance contains elements of Pavlovian conditioning, instrumental learning, and motor learning and it is possible that these can become uniquely intertwined so as to recruit different neural substrates that help to maintain a short-term aversively motivated motor memory that allows laboratory rats to exhibit inhibitory avoidance behaviour even in the face of amygdaloid NMDA receptor antagonism (see Kim and Davis, 1993a, b; Lee, et al., 1996b; Walker and Davis, 2000). As a result, it is possible that the amygdala, and NMDA receptors located within the amygdala, make different contributions to the acquisition and memory consolidation processes during and after fear training in these two paradigms (Vazdarjanova and McGaugh, 1999; also see, Maren, Aharonov, Stote, and Fanselow, 1996; Wilensky, et al., 1999; Walker and Davis, 2000).

In addition to the importance of NMDA receptors in mediating fear acquisition (Campeau, et al, 1992; Miserendino, et al., 1990; Fanselow and Kim, 1994; Walker and Davis, 2000), the inhibitory avoidance results discussed above indicate that NMDA receptors may be involved in supporting memory consolidation and retention processes

occurring after fear acquisition training has been terminated, and research has demonstrated that posttraining intra-amygdala infusion of AP5 caused a memory-impairing effect in rats trained in a one trial step-down inhibitory avoidance task (Izquierdo, Da Cunha, Rosat, Jerusalinsky, Ferreira, and Medina, 1992; Jerusalinsky, et al., 1992; Liang, Hon, and Davis, 1994). The notion that NMDA receptors are involved in fear memory consolidation processes is supported by a series of elegant experiments carried out by Liang, Hon, and Davis (1994). In these experiments Liang et al., (1994) used a one trial step-through inhibitory avoidance task to investigate the effects of pretraining, posttraining, and pretest intra-amygdalar infusion of AP5, at various doses, on avoidance retention as well as the ability of NMDA agonists to reverse the amnesic effects produced by AP5 (Liang, et al., 1994). The results of these studies found that pretraining infusion of AP5 into the amygdala produced a dose-dependent retention deficit on inhibitory avoidance, an effect that was ameliorated by immediate posttraining intra-amygdala infusion of NMDA but not NMLA the L-isomer of the racemic NMDLA (Liang, et al., 1994). Similar pretraining infusions of AP5 into the striatum and ventral hippocampus failed to cause a retention deficit, indicating that the amnesic effect that was produced by AP5 was localized to NMDA receptor antagonism in the amygdala (Liang, et al., 1994). In addition, posttraining AP5 infusion into the amygdala also caused a dose-dependent and time-dependent retention deficit with a 5.0 μg dose of AP5 producing the largest effect. This effect on retention only occurred if the AP5 was infused immediately following training since delayed infusions made five hours after avoidance training were found to produce no such deficit (Liang, et al., 1994). This time-dependent memory deficit provides solid evidence that AP5 affects memory processes occurring within the amygdala.

More recent experimental assessments on the role of NMDA amygdaloid receptors in facilitating the acquisition and formation of long-term memories for fear conditioning have been made by Walker and Davis (2000). By utilizing the FPS paradigm in a series of experiments, these researchers were able to determine that blockade of NMDA receptors in the BLA with AP5 prior to fear conditioning partially impaired the formation of short-term CS-UCS fear memories in rats when short test-train intervals were used to assess FPS (Walker and Davis, 2000). Generally speaking, this study showed that the development of FPS in AP5 infused rats during the immediate test phase was arrested or somewhat delayed

when compared to phosphate buffered saline control animals (Walker and Davis, 2000). More important was the finding that AP5 administration into the BLA completely abolished FPS responding when rats were tested 48 hours later because it seemed to indicate that NMDA receptors in the amygdala are likely involved in fear memory consolidation processes as well (Walker and Davis, 2000). Thus, it is highly probable that amygdaloid NMDA receptor blockade with AP5 and/or other similar pharmacological agents (e.g. AP7; see Miserendino, et al., 1990) may not only interfere with neural mechanisms that support fear acquisition and associative learning (i.e. the formation of CS-UCS associations) but also may disrupt post-training processes that set in motion the sequence of molecular and biochemical events that help stabilize newly formed memories and shift them from a short-term memory state into a long-term memory state (McGaugh, Introini-Collison, Cahill, Castellano, Dalmaz, Parent and Willams, 1993; Liang, et al., 1994; Walker and Davis, 2000).

The results mentioned above along with those obtained from other fear learning paradigms, seem to indicate that lateral and basolateral amygdaloid NMDA receptors are involved in generating the type of neurosynaptic transmission and synaptic plasticity that contributes to fear learning as well as memory consolidation process that occur during and after fear training. For example, pre-training infusions of NMDA receptor antagonists into the amygdala have been shown to significantly impair the long-term retention of avoidance behaviour, whilst leaving acquisition and short-term fear retention nearly intact or unaffected (Kim and McGaugh, 1992; Bianchin , Mello e Souza, Medina and Izquierdo, 1999; Roesler, Vianna, de-Paris, Rodrigues, Sant'Anna, Quevedo, and Ferreira, 2000). Moreover, pre-training infusion of AP5 into the BLA prevents fear conditioning to an auditory CS (i.e. 82 decibel tone) in rats that had previously been fear conditioned to a visual CS (i.e. light) (Lee and Kim, 1998), once again highlighting the important role played by amygdaloidal NMDA receptors in the acquisition and consolidation of new fear memories (i.e. CS-UCS associations). Also infusion of AP5 into the basolateral but not the central nucleus of the amygdala was reported to block contextual fear conditioning as measured by the defensive freezing paradigm (Fanselow and Kim, 1994), indicating that the basolateral nucleus is critically involved in fear learning processes. In a more recent study on rats, Goosens and Maren (2003) have demonstrated that basolateral amygdaloid

NMDA receptors are essential for acquiring CS-UCS fear associations during Pavlovian fear conditioning and that the amygdala may be a site where some fear memories are permanently stored. These researchers used "savings", a form of subthreshold learning that is not exhibited during an initial testing session but rather is expressed later as facilitated re-acquisition to determine whether NMDA receptor blockade in the basolateral or central nucleus of the amygdala prior to fear conditioning would affect both the acquisition and savings of auditory and contextual fear memories in rats (Goosens and Maren, 2003). The research data gathered from these experiments revealed that intra-BLA infusion of the NMDA receptor antagonist AP5 prior to auditory fear or contextual fear conditioning (i.e. tone + shock pairings or context + shock pairings) produced severe deficits in fear learning in rats when the tone or context CS was presented during behavioural testing (Goosens and Maren, 2003). Interestingly, similar deficits in fear learning were caused by pre-training AP5 microinfusions made into the central nucleus of the amygdala (Goosens and Maren, 2003).

With regards to "savings", rats infused with AP5 into the BLA prior to fear conditioning tended to reacquire conditioned fear more slowly than vehicle controls and at near identical rates as control rats that had received no prior fear conditioning, thus suggesting that there was no evidence of fear savings in rats infused with AP5. Hence, antagonism of NMDA neurons in the BLA prior to fear conditioning not only blocked fear learning as measured by freezing behaviour but also produced deficits in "savings" when rats were subsequently given additional training and testing sessions (Goosens and Maren, 2003). In contrast, rats that were microinjected with AP5 into the central nucleus of the amygdala displayed savings as they generally showed a greater rate of fear reacquisition on the first test trial administered after the first reminder fear conditioning trial (Goosens and Maren, 2003). On the whole the above results are generally consistent with previous work from Maren and associates (1996b) that demonstrated that NMDA receptors in the basolateral nucleus of the amygdala are necessary for both the acquisition and expression of contextually signalled conditioned fear in rats (Maren, Aharonov, Stote, and Fanselow, 1996b).

6.41: Amygdaloid Glutamate Receptors are Also Involved in Fear Expression and Synaptic Transmission Processes that Aid Fear Memory Retrieval

As discussed above, a plethora of experimental research suggests that NMDA amygdaloid receptors are crucial for fear acquisition and LTP processes associated with learning and memory functions in response to fear evoking stimuli (Miserendino, 1990; Campeau, et al., 1992; Gerwitz and Davis, 1997; Rogan, et al., 1997a,b; McKernan and Shinnick-Gallagher, 1997; Walker and Davis, 2000; Goosens and Maren, 2003). However, it is important to point out that NMDA receptors and/or other non-NMDA glutamatergic receptors are also involved in the retrieval processes associated with the behavioural expression of fear (Maren, Aharonov, Stote, and Fanselow, 1996; Lee and Kim, 1998; Kim, Campeau, Falls, and Davis, 1993; Fendt, 2001). For example, it has been recently reported that AP5 infusion into the lateral amygdaloid nucleus blocked the expression of FPS and defensive freezing in rats (Fendt, 2001), suggesting that in addition to facilitating LTP induction during fear learning, NMDA receptors may also be involved in glutamatergic-mediated synaptic transmission processes within the amygdala that are necessary for fear expression (Fendt, 2001; Maren, et al., 1996; Lee and Kim, 1998; Lee, Chio, Brown and Kim, 2001; Gean and Chang, 1992). Specifically, the expression of FPS was completely blocked by 12.5, 25, and 50 nano-mole doses of AP5 infused into the lateral amygdaloid nucleus, whilst the expression of defensive freezing behaviour measured through the inhibition of motor activity was attenuated in a dose-dependent fashion with the highest dose of AP5 (i.e. 50 nanomoles) being the most effective in suppressing the expression of this conditioned fear response (Fendt, et al., 2001).

Similar results on fear expression following amygdaloidal NMDA receptor blockade have been obtained by Lee and associates (2001) who employed several different measures of conditioned fear in order to assess the role played by basolateral amygdaloid receptors on fear expression. The measures chosen by these researchers included defensive freezing to an auditory or contextual conditioned stimulus, conditioned hypoalgesia measured by the tail flick latency test, conditioned ultra-sonic vocalization, and defecation levels in response to fear eliciting cues (Lee, et al., 2001). The results of this study demonstrated that infusion of the NMDA receptor antagonist AP5 into the BLA of fear conditioned rats prior to retention testing blocked the expression of both tone and contextual conditioned fear as

measured by defensive freezing behaviour (Lee, et al., 2001). In a similar fashion, AP5 infusions made into the BLA also blocked fear expression when it was assessed by the tail flick test, ultrasonic vocalization intensity test, or by calculating average defecation levels (Lee, et al., 2001). Interestingly, the Lee, et al., study (2001) also demonstrated that intra-amygdalar infusion of AP5 prior to fear conditioning impaired the acquisition of conditioned fear to both auditory and contextual cues that were paired with footshocks and experiments involving ultrasonic vocalizations closely paralleled the defensive freezing results. Furthermore, Lee and colleagues (2001) reported a high correlation between the size of postshock fear responding recorded during fear training (i.e. the immediate fear tests) and the subsequent expression of conditioned fear exhibited by rats on the retention test conducted at a much later time. Hence, AP5-infused rats that displayed an acquisition deficit during the training phase generally failed to express conditioned fear on the retention test. Lee and associates (2001) note this is consistent with the notion that pharmacological agents like AP5 disrupt fear conditioning not only by blocking amygdaloidal LTP but also by interfering with normal excitatory synaptic transmission functioning that is used to relay CS and UCS sensory information so that it can be integrated to facilitate fear learning, fear memory consolidation and retrieval, and fear expression.

Keeping this in mind, it is necessary to point out that research has demonstrated that changes in glutamatergic synaptic transmission processes involving both NMDA and AMPA kainate receptors do occur quite frequently in the amygdala and these changes are especially pronounced either during classical fear conditioning or electrical activation of the BLA (Gean and Chang, 1992; Davis, Rainnie and Cassell, 1994; Li, et al, 1995; McKernan and Shinnick-Gallagher, 1997). For example, afferents from large spine-dense pyramidal neurons of the lateral and basolateral nuclei are glutamatergic (Farb, Aoki, and LeDoux, 1995), and stimulation of the basolateral amygdaloid complex causes excitatory post synaptic potentials in the central amygdaloid nucleus which has both AMPA/kainate and NMDA receptor mediated elements (Davis, et al., 1994; Nose, Higashi, Inokuchi, and Nishi, 1991). Furthermore, Fendt (2001) points out that several electrophysiological studies have demonstrated that AP5 alters normal glutamatergic transmission within the amygdala (Gean and Chang, 1992; Li, et al., 1995; 1996; Maren and Fanselow, 1995;

Weisskopf et al., 1999) and doses of AP5 which inhibit LTP induction in the lateral nucleus of the amygdala also block glutamate transmission in the amygdala as well (Chapman and Bellavance, 1992). In contrast, doses of AP5 that reportedly did not disrupt glutamatergic transmission in the lateral amygdala also did not affect LTP induction (Chapman and Bellavance, 1992). Since it is accepted that LTP is necessary for learning and memory function, the findings that doses of AP5 sufficient to block LTP in the amygdala also block fear conditioning adds credence to the suggestion that learning and memory processes occur in the amygdala during fear conditioning. Moreover, the finding that many forms of fear acquisition and expression can be blocked by the intra-amygdalar administration of NMDA receptor antagonists lends support to the view that normal excitatory synaptic transmission and LTP-events occurring within this limbic structure make it a site that is ideally suited to accommodate and retrieve fear memories. Indeed, LeDoux (2000) and various others (Maren, 1996; Maren, 1999; Lee, et al., 2001; Fendt, 2001) have discussed these ideas in the context of a "transmission hypothesis" involving the glutamatergic transmission of auditory and visual information within the amygdala. The views these researcher express are particularly relevant given the fact that synaptic transmission involves both NMDA and AMPA receptors (Gean and Chang, 1992; Gean, Chang, Huang, Lin and Way, 1993; Li, et al., 1995; McKernan and Shinnick-Gallagher, 1997; Wang, et al., 2001; 2002) and some forms of amygdaloid LTP may not necessarily require NMDA receptor activation but rather may rely on calcium-permeable AMPA receptors formed from subunits that contain glutamine (Huang and Kandel, 1998; McKernan and Shinnick-Gallagher, 1997; Chapman and Bellavance, 1992; Siegelbaum, Schwartz and Kandel, 2000; in Kandel, Schwartz and Jessell, 2000).

6.42: The Involvement of Amygdaloidal AMPA Receptors in Fear Retrieval and Expression

Thus, in addition to the contribution of amygdaloid NMDA receptors to fear learning and expression, it quite plausible that AMPA receptors found in the BLA are involved in mediating some of these functions as well. For example, research has demonstrated that pretest infusion of the non-NMDA ionotropic AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) into the BLA dose-dependently blocked the

expression of FPS in rats that had been trained using either an auditory or visual CS (Kim, Campeau, Falls, and Davis, 1993). Furthermore, Walker and Davis (1997) studied the involvement of the BNST along with the central and basolateral amygdaloid nuclei in the expression of conditioned and unconditioned fear using both FPS and light-enhanced startle as behavioural measures (Walker and Davis, 1997b). In the latter paradigm no shock was used, but rather a high intensity bright light was employed as an anxiogenic stimulus. Exposure to a bright light has been shown to reliably enhance the acoustic startle response in rats and this increase in startle is considered to be a measure of unconditioned fear or anxiety (Walker and Davis, 1997a; Walker and Davis, 1997b). Using this method, Walker and Davis (1997b) found that infusion of the AMPA/kainate receptor antagonist 2, 3-dihydroxy-6-nitro-7-sulphamoylbenzo (F)-quinoxaline (NBQX) into the central nucleus of the amygdala blocked the expression of FPS but not light-enhanced startle. Conversely, infusion of NBQX into the BNST attenuated the expression of light-enhanced startle but not FPS (Walker and Davis, 1997b). Moreover, infusion of NBQX into the BLA was shown to block the expression of both FPS and light-enhanced startle, whereas infusions into the BNST only blocked light-enhanced startle. This result is not surprising since the BLA innervates and relays information to both the BNST and the central nucleus of the amygdala (Krettek and Price, 1978a,b; Sun, Roberts and Cassell, 1991; Alheid, et al., 1995). As such, interference with synaptic transmission via NBQX infusion into the BLA prevents the expression of FPS and light-enhanced startle (Walker and Davis, 1997b). This seems to suggest that AMPA receptors in the BLA are important for fear memory retrieval, conditioned fear expression and anxiety-motivated reflexive responding.

Similarly, pretest infusions of CNQX into the amygdala were also shown to impair the performance of inhibitory avoidance on a retention test when this drug was administered one day after fear training, however when pretest infusions where made 21 days after fear conditioning CNQX was shown to be less effective in blocking inhibitory avoidance (Liang, 1991). Nevertheless, other research groups who have studied the effects of CNQX on fear expression measured by inhibitory avoidance have found that intra-amygdaloid application of this AMPA receptor blocker effectively disrupts the performance of this fear-motivated response (Izquierdo, Bianchin, Bueno E Silva, Zanatta, Walz, Ruschel, Da Silva, Paczko and Medina, 1993). Furthermore, post-training infusions of CNQX (i.e. intra-

amygdalar) either immediately after training or up to three hours after training has been terminated causes a pronounced disruption in inhibitory avoidance behaviour during retention testing (Jerusalinsky, Ferreira, Walz, DaSilva, Bianchin, Ruschel, Median and Izquierdo, 1992; Izquierdo, et al., 1993) indicating that interference with AMPA-receptor-mediated synaptic transmission processes does impair fear-motivated learning. Also, Maren (1999) reports that the expression of conditioned freezing was attenuated by infusions of CNQX into the basolateral nucleus of the amygdala indicating that AMPA receptor antagonists interfere with synaptic transmission processes that may be vital for fear-memory retrieval.

In stark contrast, pre-treatment of laboratory animals with AMPA receptor agonists has been shown to enhance the acquisition of fear conditioning (Rogan, Stäubli and LeDoux, 1997b). This indicates that AMPA/kainate receptors may be involved in synaptic transmission processes that assist in the retrieval of fear memories and in the expression of behaviours that are typically associated with a central fear-state (Gean and Chang, 1992; Li, Phillips and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997; Maren, 1999; Rogan, et al., 1997b). This notion is supported by the fact that several studies have demonstrated that AMPA receptors contribute to synaptic transmission in pathways that impinge on the lateral and basolateral amygdaloid nuclei (Gean and Chang, 1992; Li, et al., 1995; McKernan and Shinnick-Gallagher, 1997). Thus, the activation of BLA AMPA receptors with AMPA receptor agonists may facilitate fear conditioning and fear expression. This seems quite plausible as blockade of AMPA receptors in the BLA with NBQX does interfere with fear-memory retrieval, FPS expression, and anxiety-enhanced acoustic startle responses (Walker and Davis, 1997b).

6.43: The Role of Amygdaloidal Metabotropic Glutamate Receptors and Voltage-Gated Calcium Channels in Fear Learning, Fear Memory Consolidation and Fear Expression

While much attention has focused on the role of amygdaloidal NMDA and AMPA receptors in mediating such events as LTP, fear learning, memory consolidation, and fear expression, it should be noted that recent research work has also evaluated the role of metabotropic glutamate receptors (mGluRs) and voltage-gated calcium channels (VGCCs) in this capacity (Masugi, Yokoi, Shigemoto, Muguruma, Watanabe, Sansig, van der Putten

and Nakanishi, 1999; Lee, Lee, and Choi, 2002; Fendt and Schmid, 2002; Walker, Rattiner and Davis, 2002b; Bauer, Schafe and LeDoux, 2002; Rodrigues, Bauer, Farb, Schafe, and LeDoux, 2002; Shinnick-Gallagher, McKernan, Xie, Zinebi, 2003). In fact several scientific studies have demonstrated that mGluRs play a prominent role in the acquisition of conditioned fear (Rodrigues, et al., 2002; Masugi, et al., 1999; Bianchin, Spanis, Roesler, McGaugh, and Izquierdo, 2000; Fendt and Schmid, 2002; Walker, et al., 2002b). For example, systemic administration of the metabotropic glutamate receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) has been shown to block conditioned fear in rats (Schulz, Fendt, Gasparini, Lingenhöhl, Kuhn, and Koch, 2001). More recent research has shown that discrete infusions of MPEP into the lateral nucleus of the amygdala (0.001, 010, 0.100, and 1.000 μgs/0.5 μl) dose-dependently prevented the acquisition of FPS with the three highest doses of this antagonist being the most effective (Fendt and Schmid, 2002). In addition, the 1.000 μg dose of MPEP also blocked defensive freezing behaviour measured by the inhibition of motor activity (Fendt and Schmid, 2002). Thus, pre-training intra-amygdala infusions of this particular metabotropic receptor antagonist prevented fear learning when rats were tested for conditioned fear 48 hours later. In contrast, pretest infusions of MPEP using the same doses mentioned above failed to block the expression of either FPS or fear-induced freezing behaviour. Furthermore, microinfusion of MPEP (0.01 μg dose) into the lateral amygdaloid nucleus immediately after fear conditioning did not seem to impair the consolidation of conditioned fear when rats were tested for FPS 48 hours later during a retention test. These data seem to indicate that blockade of the mGluR5 receptor subtype with MPEP disrupts acquisition but not the consolidation or expression of conditioned fear (Fendt and Schmid, 2002).

These results are highly consistent with the findings reported by Rodrigues and colleagues (2002) who also demonstrated that infusion of MPEP into the lateral nucleus of the amygdala blocks the acquisition but not the expression or consolidation of auditory and contextual fear conditioning. More specifically, these researchers revealed that 0.15 and 1.5 μg doses microinjected into the lateral amygdaloid nucleus prior to fear conditioning significantly impaired short-term auditory and contextual fear memories when rats were assessed for freezing behaviour 1 hour after fear conditioning. Long-term auditory and contextual fear memories were also disrupted by MPEP application into the lateral

amygdala as rats that exhibited short-term fear memory deficits measured one hour after fear conditioning also displayed similar deficits in fear-induced freezing behaviour when administered the retention test 24 hours later (Rodrigues, et al., 2002). Not surprisingly, MPEP intra-amygdalar infusions made either immediately after fear conditioning or just before retention testing did not significantly attenuate memory consolidation or the expression of conditioned fear to auditory and contextual conditioned stimuli (Rodrigues, et al., 2002), indicating that the blockade of amygdaloid mGluR5 receptors with MPEP acts to specifically interfere with the acquisition phase of Pavlovian fear conditioning.

Furthermore, L-LTP induction at thalamo-amygdaloid synapses was prevented by either the mGluR antagonist MPEP (10 μM) or the NMDA receptor antagonist AP5 (50 μM) when each compound was applied separately to brain slice preparations before or during tetanus administration (Lee, Lee, and Chio, 2002). When these antagonists were applied after LTP had been firmly established in the thalamo-amygdala pathway, they could not block the expression of LTP (Lee, et al., 2002). It is important to point out that in both the Fendt and Schmid (2002) and the Rodrigues, et al., (2002) studies LTP induction (in vitro) in lateral amygdaloid nucleus was also significantly impaired by MPEP application to this particular amygdalar nuclear region. These results once again provide more compelling electrophysiological evidence that thalamo-amygdalar circuits and glutamate receptors (i.e. mGluRs and NMDA receptors) have a critical role in facilitating long-term synaptic changes that enable the amygdala to become intimately involved in fear learning, memory consolidation and fear expression.

Further research highlighting the importance of amygdaloid mGluRs in mediating fear responses comes from studies of mGluR7$^{-/-}$ knock out mice that lack mGluR7 receptors. This research showed that the mGluR7$^{-/-}$ knock out mice exhibited considerably less defensive freezing behaviour during context + footshock presentations than the genetically unaltered wild mice that served as the experimental control group (Masugi, et al., 1999). A retention test of contextual fear administered 24 hours after exposure to 10 footshocks in the experimental chamber indicated that the mGluR7$^{-/-}$ knock out mice had profound impairments in both consolidating and expressing contextual fear since these mice were observed to freeze significantly less to contextual cues than the wild-type control mice (Masugi, et al., 1999). Additionally, mGluR7$^{-/-}$ knock out mice also exhibited impairments

in conditioned taste aversion learning, a form of learning that appears to rely heavily on the amygdala and its interactions with the parabrachial nucleus and the gustatory cortex (Yamamoto, Shimura, Sako, Yasoshima and Sakai, 1994). Furthermore, immunoreactive staining of the BLA revealed that the mGluR7$^{-/-}$ mice exhibited far less immunoreactive neuronal tissue used to indicate the presence of mGluRs than the wild control mice (Masugi, et al., 1999). This in combination with the behavioural results reported above adds support for the notion that mGluRs in the basolateral amygdaloid complex are involved in helping to establish CS-UCS fear associations during Pavlovian fear conditioning. This notion is further supported by the fact that microinfusion of metabotropic glutamate receptor antagonists (i.e. {±}-Alpha-methyl-4-carboxyphenylglycine) into the basolateral nucleus of the amygdala has been shown to attenuate inhibitory avoidance behaviour during retention testing (Bianchin, et al., 2000).

More recently, group II metabotropic glutamate receptor activation in the BLA has also been shown to disrupt fear learning and expression in a FPS paradigm (Walker, Rattiner and Davis, 2002b). For example, the expression of FPS was completely blocked by intra-BLA infusions of 0.3 and 1.0 μg per side doses of the metabotropic group II receptor agonist LY354740 prior to fear testing and it was reported that this effect on fear expression could be reversed by pre-treatment with LY341495 which is a Group II metabotropic receptor antagonist (Walker, et al., 2002b). More specifically, infusions of LY354740 (0.3 μg/per side) into the BLA prior to fear conditioning, retention testing or both effectively attenuated FPS responding, indicating that both fear acquisition and expression (i.e. performance) was affected by this Group II metabotropic receptor agonist (Walker, et al., 2002b). Also, any impairment in fear expression caused by LY354740 could not be attributed to amygdaloid damage or neurotoxic effects associated with the drug because rats that previously received LY354740 before final testing exhibited levels of FPS that were similar in magnitude to PBS controls when retested 10 days later in a non-drug state. In addition, infusion with APDC a second Group II metabotropic agonist produced similar effects on FPS expression to those caused by LY354740, indicating that the action of LY354740 on fear was likely attributable to activation of Group II metabotropic receptors in the basolateral complex (Walker, et al., 2002b). Walker and colleagues (2002b) speculate that the activation of Group II metabotropic glutamate

receptors likely blocks conditioned fear either by hyperpolarizing BLA neurons or diminishing synaptic transmission between the lateral and basolateral nuclei of the amygdala. According to this view activation of Group II metabotropic glutamate receptors may inhibit amygdaloidal function through the inhibitory actions of postsynaptic receptors or by interrupting synaptic transmission in the lateral to basolateral pathway at the level of presynaptic receptors (Walker, et al., 2002b; also see Holmes, Bradley, Arvanov, and Shinnick-Gallagher, 1996; Patil and Rainnie, 2000; Neugebauer, Keele, and Shinnick-Gallagher, 1997). Activation of amygdaloidal Group II metabotropic glutamate receptors by Group II metabotropic agonists hinders amygdaloid functioning in a mechanistically different fashion to metabotropic receptor antagonists such as MPEP that specifically target the mGluR5 receptor subtype that belongs to the Group I category of mGluRs. However, it can be stated with some degree of certainty that Group I and Group II metabotropic glutamate receptors are necessary for fear learning (see Rodrigues, et al., 2002; Fendt and Schmid, 2002; Walker, et al., 2002b). At present, however Group II mGluRs seem to be critically involved in both the acquisition and expression of FPS (Walker, et al., 2002b). In contrast, the Group I and Group III family of mGluRs (i.e. mGluR5, mGluR7 subtypes) seem to be more involved in fear acquisition and less involved in fear expression (Fendt and Schmid, 2002; Rodrigues, et al., 2002; Masugi, et al., 1999).

6.44: L-Type Voltage Gated Calcium Channels May Be Involved in Long-Term Fear Memory Consolidation

As was mentioned to earlier, tetanic stimulation of either thalamic or cortical afferents to the lateral amygdala can result in an NMDA-independent form of amygdaloidal LTP that is exclusively mediated through L-type voltage-gated calcium channels (Chapman and Bellavance, 1992; Huang and Kandel, 1998; Li, Weiss, Chuang, Post, and Rogawski, 1998; Weisskopf, Bauer, and LeDoux, 1999; Bauer, Schafe and LeDoux, 2002). Recent research efforts have demonstrated that administration of the L-type voltage-gated calcium channel blocker, nifedipine, prevents the induction of amygdaloid LTP produced by the coupling of weak presynaptic stimulations of thalamo-amygdalar afferents with strong postsynaptic depolarization triggered by current injections made into the postsynaptic cell (Wiesskopf, et al., 1999; Schafe, et al., 2000; Bauer, et al., 2002). Similar results have also been achieved

with the L-type voltage-gated calcium channel blocker verapamil, in that it too prevents LTP induction when a presynaptic-postsynaptic coupling protocol is employed during LTP induction in the lateral nucleus of the amygdala (Bauer, et al., 2002). These findings have caused some researchers to propose that L-type voltage-gated calcium channels found in the lateral and basolateral nuclei of the amygdala may be involved in fear learning and fear memory consolidation processes (Schafe, et al., 2001; Bauer, et al., 2002; Shinnick-Gallagher, et al., 2003).

Indeed, recent electrophysiological and pharmacological manipulations of the lateral amygdala have made significant progress towards elucidating the role played by L-type voltage-gated calcium channels in mediating conditioned fear-memory formation (Bauer, et al., 2002; Shinnick-Gallagher, et al., 2003). For example, intra-lateral amygdaloid nucleus infusions of verapamil (0.04 μg, 0.4 μg, or 4.0 μg dose per hemisphere) prior to Pavlovian auditory fear conditioning produced no noticeable deficits in immediate postshock defensive freezing behaviour at any dose tested, however, verapamil did cause a significant dose-dependent impairment in long-term fear memory to an auditory CS when rats were assessed for freezing behaviour twenty-four hours later (Bauer, et al., 2002). More specifically, the 0.4 and 4.0 μg doses were most effective in causing a significant disruption in long-term fear memory formation as the level of defensive freezing recorded during the retention test was significantly reduced in the verapamil-treated rats relative to the vehicle control group administered dH_2O prior to auditory fear conditioning (Bauer, et al., 2002). Consistent with this finding is the fact that a 4.0 μg dose of verapamil reportedly did not impair the short-term memory of conditioned fear when rats were tested at 1, 3, and 6 hour intervals after fear conditioning, but it did block long-term fear memory consolidation when these rats were tested 24 hours after intra-amygdala drug infusion and auditory fear conditioning (Bauer, et al., 2002). Thus, blockade of L-type voltage-gated calcium channels by verapamil infusion into the lateral nucleus of the amygdala before tone + shock fear conditioning trials seemed to cause profound impairments in fear memory consolidation as rats infused with this particular calcium channel blocker did not exhibit high levels of CS-elicited freezing behaviour when compared to control animals (Bauer, et al., 2002). Interestingly, Bauer and colleagues (2002) also demonstrated that intra-amygdalar infusions using the NMDA receptor antagonist AP5 impaired short-term fear

memory (i.e. acquisition) at all short-term testing intervals (i.e. 1, 3, 6 hours) as well as long-term fear memory when rats were assessed for freezing behaviour at the twenty-four hours mark (Bauer, et al., 2002). These results have led this group of research scientists to the conclusion that both L-type voltage-gated calcium channels and NMDA receptors are crucial for fear memory consolidation albeit in qualitatively different ways (Bauer, et al., 2002).

For instance, Bauer and associates (2002) posit that voltage-gated calcium channels in the amygdala's lateral nucleus may be more involved in long-term fear memory formation, whilst NMDA receptors in this region likely contribute to the establishment of short-term fear memories. These researchers cite scientific evidence which demonstrates that NMDA receptors and the NR2B subunit seem to be involved in the establishment of short-term fear memories (Bauer, et al., 2002; also see Walker and Davis, 2000; Rodrigues, et al., 2001). It has also been suggested that NMDA receptor activation and the influx of calcium during fear conditioning may activate local protein kinases that in turn phosphorylate existing membrane proteins that lead to the formation of short-term fear memories (Bauer, et al., 2002; Soderling and Derkach, 2000). In stark contrast, activation of L-type voltage gated calcium channels may result in calcium-mediated events that lead to the activation of several protein kinases (e.g. PKA, MAPK) that can migrate to the cell nucleus to trigger CREB/CRE-induced gene expression and the synthesis of new mRNA and proteins that are critically involved in facilitating the establishment of long-term memories (Blair, Schafe, Bauer, Rodrigues and LeDoux, 2001; Schafe, et al., 2001; Bauer, et al., 2002; Bailey, et al., 1999; Schafe, et al., 2000; Schafe and LeDoux, 2000).

In fact there are several lines of research evidence that tend to support the occurrence of such biochemical events. First, it has recently been shown that L-type voltage-gated calcium channels, can through the activation of certain elements of the cAMP-PKA-MAPK-CREB signalling pathway direct CRE to stimulate gene transcription and protein synthesis (Dolmetsch, Pajvani, Fife, Spotts, and Greenberg, 2001). Second, it is well established that introducing compounds into the amygdala that interfere with MAPK, Ca^{2+}MII, Ca^{2+}MKIV, CREB, and new mRNA generally disrupts long-term but not short-term fear memory (Schafe, et al., 2000; Josselyn, et al., 2001). Third, behavioural assessments carried out on $CaMKIV^{-/-}$ knock out mice that exhibit no CREB activation

after fear conditioning has demonstrated that these animals tend to have significant difficulties in consolidating fear memories (Wei, et al., 2002).

Thus, L-type voltage-gated calcium channels appear to play a prominent role in helping to establish enduring fear memories and recent electrophysiological and behavioural studies carried out by Shinnick-Gallagher and colleagues (2003) seems to provide additional support for this view. For example, intraperitoneal injections of nimodipine (an L-type calcium channel antagonist; 1.5 mg/kg, 2.5 mg/kg, 10 mg/kg, 20 mg/kg) fifteen minutes before final testing were shown to block the expression of FPS in a dose-dependent fashion with the two highest doses being the most effective (Shinnick-Gallagher, McKernan, Xie, and Zinebi, 2003). Physiologically, nimodipine had an excitatory effect on post synaptic currents (EPSCs) generated in slice preparations of lateral amygdaloid neurons taken from rats that had been exposed to strict Pavlovian fear conditioning. Generally speaking, neurons obtained from fear conditioned rats typically exhibit augmented EPSCs when compared to unpaired controls or experimentally naïve animals (McKernan and Shinnick-Gallagher, 1997; Shinnick-Gallagher, et al., 2003). However, when nimodipine (10 μM dose) was introduced to the neurons obtained from fear conditioned animals it caused a significant reduction in the amplitude of EPSCs and seemed to depress synaptic transmission as well (Shinnick-Gallagher, et al., 2003). This depressing effect on synaptic transmission was ascertained by an examination of the slopes of the input-output functional relationship for EPSCs in fear conditioned, naïve, and unpaired control rats and this analysis revealed that nimodipine (10 μM) application significantly reduced the slope of the input-output curve that could be obtained from lateral amygdaloid neurons taken from fear conditioned animals (Shinnick-Gallagher, et al., 2003). Interestingly nimodipine had no effect on the input-output relationship data gathered from naïve rats which seems to suggest that L-type voltage-gated calcium channels are not usually involved in normal synaptic transmission in thalamo-amygdalar pathways when animals are not challenged by aversive environmental stimuli or fear conditioning trials that require some learning (Shinnick-Gallagher, et al., 2003). Nevertheless, it is conceivable that once animals are exposed to aversive stimuli during fear conditioning or have in the past experienced such events, L-type voltage-gated channels may be more active in assisting with synaptic transmission processes that are mediated by NMDA and AMPA glutamate receptors, but this possibility

still needs to be scientifically evaluated. In any event, the information presented in the above discussions seems to strongly suggest that L-type voltage-gated calcium channels in the lateral nucleus of the amygdala play a pivotal role in LTP induction and in the consolidation of conditioned fear. Discussions in the remaining sections of this chapter will now shift focus in order to briefly examine how amygdaloid benzodiazepine and GABA receptor activation inhibits fear learning and fear expression before moving on to provide a detailed account of the contribution made by the mesolimbic DA system and amygdaloid DA receptors in fear learning and expression.

6.5: Benzodiazepine and GABAergic Amygdaloid Receptors and Conditioned Fear

Traditionally, benzodiazepine drugs (e.g. chlordiazepoxide, diazepam) have been used as a pharmacological intervention for managing fear and anxiety disorders in humans (File, 1987; Wardle, 1990; Lader, 1994; Lader, 1999). Although benzodiazepines are highly effective in reducing anxiety and fearfulness in most patients, they are also highly addictive and often cause several undesirable side effects such as drowsiness, muscle relaxation, memory impairment, unpleasant withdrawal symptoms and a recurrence of anxiety once the treatment program is discontinued (Taylor, Riblet, Stanton Eison, Eison, and Temple, 1982; Lister, 1985; Lader, 1994; Lader, 1999). Because benzodiazepines were so effective in ameliorating anxiety but still had undesirable side effects researchers began to use animal models of fear and anxiety in order to investigate brain systems that were believed to contribute to fear and anxiety with a view to develop more efficacious drug treatments without the negative side effects. As a matter of fact ever since benzodiazepine receptor sites were detected in the central nervous system (Squires and Brastrup, 1977; Möhler and Okada, 1997) scientific efforts have focused on the amygdala and other limbic brain areas (e.g. septum, hypothalamus and hippocampus) that have been implicated in generating highly charged emotional reactions (Hess, 1936; Papez, 1937; King, 1958; King and Meyer, 1958; MacLean, 1949;1952; 1958; Schwartbaum and Gay, 1966; Fried, 1973; Gray, 1982; Gray and McNaughton, 1983; Melia and Davis, 1991; Davis, 1992a,b,c; Pesold and Treit, 1995; Treit and Menard, 1997).

Radiolabeling studies have found very high concentrations of benzodiazepine receptors in the basolateral amygdaloid complex (Young and Kuhar, 1980; Neihoff and Kuhar, 1983;

Thomas, Lewis, and Iversen, 1985; Richards and Möhler, 1984) and throughout various other limbic regions of the brain (Young, Neihoff, Kuhar, Beer, and Lippa, 1981; Richards and Möhler, 1984; Young and Kuhar, 1980). Not surprisingly, research has shown that the BLA is particularly sensitive to the anxiolytic effects produced by benzodiazepine pharmacological agents (Petersen, Braestrup and Scheel-Kruger, 1985; Scheel-Kruger and Petersen, 1982; Shibata, Yamashita, Yamamoto, Ozaki, and Ueki, 1989; Pesold and Treit, 1994; Helmstetter, 1993; Pesold and Treit, 1995; Sanders and Shekhar, 1995; Harris and Westbrook, 1998). Indeed, several paradigms that have probed into the neural substrates that are believed to be involved in mediating conditioned fear and anxiety responses have established that intra-amygdalar infusion of benzodiazepine drugs attenuate a high proportion of behavioural correlates that occur during heightened states of fear or anxiety (Petersen, et al., 1985; Shibata, et al., 1989; Pesold and Treit, 1994; 1995; Helmstetter, 1993; Harris and Westbrook, 1995; 1998). For example, intra-amygdala infusion of diazepam (5, 10 or 20 μg dose) caused a dose-dependent increase in punished responding by significantly increasing the number of shocks rats would subject themselves to in a five minute testing period in order to drink from an electrified spout (Shibata, et al., 1989). The significant anxiolytic effect produced by the 20 μg dose of diazepam during the water-lick suppression test was reversed by a systemic pre-treatment of either the benzodiazepine antagonist Ro15-1788 (i.e. 20.0 mg/kg) or the benzodiazepine inverse agonist β-CCM (i.e. 5.0 mg/kg) (Shibata, et al., 1989). Furthermore, midalozam infusions into the rostral basolateral and lateral amygdaloid nuclei also reportedly lead to an increase in anti-conflict responding which is consistent with the notion that the numerous benzodiazepine receptors found in these two regions are likely responsible for reducing fear and anxiety responses that are provoked by aversive stimuli (Scheel-Kruger and Petersen, 1982; Petersen, et al., 1985). Similarly, a 15 μg dose of midazolam infused into the basolateral nucleus of the amygdala significantly increased the number of entries rats made into an anxiety producing open-armed elevated plus-maze (Pesold and Treit, 1995). This benzodiazepine agonist also substantially increased the amount of time rats spent in the elevated plus maze but did not impair shock-probe avoidance behaviour (Pesold and Treit, 1995). Conversely, infusions of midazolam into the central nucleus of the amygdala failed to disrupt avoidance behaviour on the open-arm elevated plus-maze but did manage to significantly impair

shock-probe avoidance responses in rats (Pesold and Treit, 1995). More importantly, the anxiolytic effects produced by intra-amygdalar midazolam application were consistently blocked by pre-infusion of the benzodiazepine receptor antagonist Ro 15-1788 into the amygdala (Pesold and Treit, 1995) thus providing strong experimental evidence that benzodiazepine amygdaloid receptor activation is required to alleviate fear and anxiety-like responses when animals encounter aversive environmental stimuli (Pesold and Treit, 1995; 1994).

6.51: Benzodiazepine Agonists and Conditioned Fear

In terms of conditioned fear, diazepam infusions (30.0 μg) into the basolateral nuclear region of the amygdala blocked both hypoalgesia and defensive freezing behaviour when rats were exposed to contextual cues that had been previously paired with aversive footshocks (Helmstetter, 1993). Diazepam infusion into the central nucleus also attenuated defensive freezing behaviour but was generally ineffective in reducing conditioned hypoalgesia in the same research animals (Helmstetter, 1993). Helmstetter (1993) suggests that this different result may be due to the fact that the basolateral amygdaloid complex has many more benzodiazepine binding sites than the central nucleus of the amygdala. This postulation seems logical since weak anxiolytic effects have been reported when cannulae are situated near the lateral portion of the central amygdaloid nucleus or in more medial portions of this nucleus (Scheel-Kruger, and Petersen, 1982; Helmstetter, 1993). More support for this notion comes from recent work conducted by Harris and Westbrook (1995; 1998) who have demonstrated that intra-basolateral amygdaloid application of benzodiazepine agonists reduce conditioned hypoalgesia and block defensive freezing behaviour in rats (see Harris and Westbrook, 1995; 1998).

In some instances, benzodiazepines not only attenuate FPS but also cause marked reductions in acoustic startle reflexive responding in both human and animal subjects, and these findings are generally consistent with ability of benzodiazepines to act centrally as effective hypnotic-sedatives that are capable of producing anxiolytic effects (Joordens, Hijzen, Peeters, and Oliver, 1997; Rodriguez-Fornells, Riba, Gironell, Kulisevsky, and Barbanoj, 1999; Ashton, 1994). For instance systemic administration of benzodiazepine drugs (e.g. diazepam, flurazepam, chlordiazepoxide, alprazolam) have been shown to block

the expression of FPS in rats as well as humans (Davis, 1979; Hijzen and Slangen, 1989; Hijzen Houtzager, Joordens, Olivier, and Slangen, 1995; Patrick, Berthot, and Moore, 1996) and these anxiolytic effects in rats can be reversed by intraperitoneal injection of the inverse benzodiazepine agonist DMCM (methyl-6-,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate) (Hijzen and Slangen, 1989). Furthermore, systemic administration of diazepam prior to contextual fear conditioning was recently observed to block freezing behaviour to contextual cues, reduce postshock fear and anxiety-like behaviour, and cause significant reductions in the mRNA expression of the immediate early gene (EGR-1) in fear conditioned rats relative to vehicle controls (Malkani, and Rosen, 2000).

These pharmacological and anxiolytic properties of benzodiazepine compounds combined with the fact that benzodiazepine receptors are so plentiful in the BLA make it highly probable that amygdaloidal benzodiazepine receptor activation suppresses several forms of fear and anxiety expression. This is quite logical given the fact that central nervous system benzodiazepine receptors are often coupled to $GABA_A$ chlorine channels on neurons (Richards and Möhler, 1984) and therefore may act to facilitate inhibition in various limbic brain regions that mediate fear expression, possibly by helping to hyperpolarize neurons in these regions through the activation of the ionotropic $GABA_A$ receptor complex which permits the entry of chloride ions (Cl^-) into cells (Mehta and Ticku, 1999; Kandel and Siegelbaum, 2000).

Generally speaking, benzodiazepine agonists such as diazepam or flurazepam tend to increase the coupling between GABA and chlorine channels (Richards and Möhler, 1984), whereas benzodiazepine antagonists (e.g. Ro15-1788) may significantly reduce this coupling. Furthermore, GABA or GABA agonists (e.g. muscimol) increase the opening of the chlorine channels on the $GABA_A$ receptor complex and permit the influx of Cl^- into the neuron (Tallman and Gallagher, 1985; Kakueff and Nutt, 1997; Kandel and Siegelbaum, 2000). This increase in intracellular Cl^- leads to membrane hyper-polarization, inhibition, and the generation of inhibitory postsynaptic potentials (IPSPs) that usually disrupts or short circuits any excitatory signal travelling within the neuron (Tallmann and Gallagher, 1985; Rainnie, Asprodini and Shinnick-Gallagher, 1991b; Kandel and Siegalbaum, 2000). Although, benzodiazepine agonists are unable to open the chloride channel directly, they still can enhance chloride channel opening and amplify inhibitory effects since GABA and

benzodiazepine will bind more strongly and for longer periods of time when both compounds are present and simultaneously active (Tallmann, Thomas, and Gallagher, 1978; Kalueff and Nutt, 1997; Arguropoulos and Nutt, 1999; Stephens, Schneider, Kehr, Jensen, Petersen and Honore, 1987; Kandel and Siegelbaum, 2000; Kandel 2000). Hence, the above discussions strongly suggest that central benzodiazepine receptor activation via benzodiazepine agonist treatment (e.g. diazepam) could possibly reduce fear and anxiety by increasing the GABA receptor complex's affinity for GABA. This seems plausible since the protein molecule of the $GABA_A$ receptor is allosteric in nature and generally is made up of the three distinct subunits (α, β, γ) that act as separate binding sites for GABA, barbiturates, and benzodiazepines respectively (Bormann, 1988; Prichett, Sontheimer, Gorman, and Kettermann, 1988; Prichett, Sontheimer, Shivers, Ymer, Kettermann, Schofield and Seeburg, 1989; Mehta and Ticku, 1999; Kandel, 2000 in Kandel Schwartz and Jessell, 2000).

It is important to point out that $GABA_A$ receptors are very complex pentamerically assembled molecules that can be made up of different combinations of the three subunits mentioned above (i.e. α, β, γ), however the most common or preferred pentameric arrangement includes a receptor molecule that is made up of either two α, two γ, and one β sub-unit or two α, two β, and one γ subunit (Mehata and Tucku, 1999; Massoti, et al., 1991; Siegelbaum and Kandel, 2000). Binding of benzodiazepine drugs onto the γ subunit for example, will typically result in stronger binding of GABA to the α-subunit and thus increase Cl^- influx and the inhibitory effects produced by the $GABA_A$ receptor complex. Thus, the anxiolytic effect created in animals exposed to fearful stimuli following benzodiazepine infusion into the amygdala can be best explained by the action these pharmacological agents have in facilitating inhibitory neurotransmission through the $GABA_A$ receptor complex (Stevens, et al., 1987; Marczynski and Urbancic, 1988; Kandel, 2000; Malkani and Rosen, 2000).

However, the ability of benzodiazepine drugs to attenuate fear and anxiety responses may depend on such factors as the potency of the benzodiazepine and the actual composition of the $GABA_A$ receptor itself as research has shown that this receptor complex can be made up of various combinations α, β, and γ subunits (Massotti, Schlichting, Antoacci, Giusti, Memo, Costa, and Guidotti, 1991; Pritchett, et al., 1988; Pritchett, et al.,

1989; Levitan, Schofield, Burt, Rhee, Wisden, Kohler, Fujita, Rodriguez, Stephenson, Darlison, Barnard, and Seeburg, 1988; Shivers, Killish, Sprengel, Sontheimer, Kohler, Schofield, and Seeburg, 1989; Mehta and Ticku, 1999). Other factors that could conceivably determine the efficiency of benzodiazepine drugs to alleviate fear and anxiety in animals include; the brain area under investigation, the actual number of benzodiazepine binding sites in a given region and the behavioural measure used to assess fear and anxiety. With regards to brain regions, some limbic areas other than the amygdala and even some midbrain regions have been shown to produce anxiolytic effects after infusions with benzodiazepine drugs (Gifkins, et al., 2002). For example, application of flurazepam (60 μg and 120 μg doses) into the VTA of rats has been shown to attenuate the shock sensitisation of startle normally observed after a series of rapid footshocks. Additionally, intra-septal infusion of midazolam administered to rats was reported to increase activity in the elevated plus-maze and cause marked reductions in shock probe burying behaviour (Pesold and Treit, 1994; 1996) suggesting that benzodiazepine binding sites in the septal nucleus may also be involved in alleviating certain types of fear or anxiety-based responses. This notion is supported by the fact that septal lesions do not interfere with diazepam's ability to block FPS (Melia and Davis, 1991) but do produce anxiolytic effects in other behavioural tasks that are used to measure fear or anxiety (see Decker, et al., 1995). For the most part however, benzodiazepine infusion into the amygdala has been shown to block conditioned defensive freezing behaviour (Helmstetter, 1993; Harris and Westbrook, 1998), conditioned hypoalgesia (Helmstetter, 1993), punished responding (Shibata, et al., 1989) and shock probe avoidance (Pesold and Treit, 1994) which indicates that benzodiazepine sites within the amygdala influence fear learning and expression as well as anxiety-like behaviours. In any event, it is quite clear that benzodiazepine receptors play a critical role in alleviating behavioural correlates of fear and anxiety but this may depend on GABAergic receptor mediated events that involve biochemical processes taking place at the α subunit of the $GABA_A$ receptor complex.

6.6: The Role of GABA receptors and GABA receptor agonists in Attenuating Conditioned Fear

It is well established that GABA behaves as the main inhibitory neurotransmitter in the central nervous system of many organisms. Thus, GABA's primary function is to inhibit, regulate, or sculpt excitatory synaptic transmission so as to ensure that the brain is not overwhelmed with the vast quantities of sensory information (Marczynski and Urbancic, 1988; Mehta, and Ticku, 1999; Kalueff and Nutt, 1997; Kandel, 2000; Siegelbaum and Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). This makes sense as immunohistochemical, anatomical, and morphological studies have revealed that numerous GABAergic non-pyramidal neurons make intimate synaptic connections with many of the pyramidal-shaped and spine-dense glutamatergic neurons found in the BLA complex (McDonald, 1985; McDonald, Beitz, Larson, Kuriyama, Sellitto, and Madl, 1989; McDonald and Pearson, 1989; Sun and Cassell, 1993; Sun, Yi, and Cassell, 1994). Also, electrophysiological studies have shown that GABA receptors in the BLA are involved in facilitating inhibitory synaptic transmission within this limbic brain region (Rainnie, Asprodini, and Shinnick-Gallagher, 1991b; Yamada, Saitow, Satake, Kiyohara, and Konishi, 1999). For example, activation of the $GABA_B$ receptor complex with the $GABA_B$ receptor agonist baclofen has been shown to inhibit fast excitatory post synaptic currents (EPSCs) in BLA brain slices induced by microelectrode stimulation (Yamdada, et al., 1999). Furthermore, baclofen (0.3 to 30 μM) introduced into the BLA by superfusion techniques significantly reduced $GABA_A$ receptor-mediated inhibitory post synaptic currents as well (Yamada, et al., 1999) and similar electrophysiological results have been obtained when the $GABA_A$ receptor antagonist bicuculline was used in this capacity (Rainnie, Asprodini and Shinnick-Gallagher, 1991; Washburn and Moises, 1992). The inhibitory effect produced by baclofen was blocked by the administration of the $GABA_B$ receptor antagonist CGP55845A and it has been suggested that baclofen may presynaptically inhibit excitatory and inhibitory synaptic inputs to the BLA by its ability to interact with autoreceptors found in glutamatergic and GABAergic terminals (Yamada, et al., 1999). However there is some indication that $GABA_B$ receptors probably influence synaptic transmission in the BLA via both a presynaptic and postsynaptic mechanism (see Rainnie, et al., 1991b). Competitive $GABA_A$ antagonists such as bicuculline may interfere

with inhibitory post synaptic currents by binding to the GABA binding site located on the α subunit and as a result prevent GABA from directly opening chlorine channels (Rainnie, et al., 1991b; Wang, Wilson, and Moore, 2001).

Electrophysiological and pharmacological studies provide compelling evidence that GABA likely mediates its inhibitory effects on fear learning and expression through the vast quantities of GABA receptors (i.e. $GABA_A$ and $GABA_B$ receptor types) located on neurons in the amygdala (Yamada, et al., 1999; Rainnie, et al., 1991b; Wang, et al., 2001; Helmstetter and Bellgowan, 1994; Muller, Corodimas, Fridel, and LeDoux, 1997; Brioni, Nagahara, and McGaugh, 1989; Izquierdo, Quillfeldt, Zanatta, Quevedo, Schaeffer, Schmitz and Medina, 1997; Castellano, Brioni, Nagahara and McGaugh, 1989; Dickinson-Anson and McGaugh, 1997; Wilensky, Schafe, and LeDoux, 1999; Wilensky, Schafe, and LeDoux, 2000; Maren, Yap, and Goosens, 2001). As a matter of fact, several pharmacologically based studies have demonstrated that GABA receptor-mediated events play a prominent role in fear acquisition, expression, and memory consolidation (Helmstetter and Bellgowan, 1994; Muller, Corodimas, Fridel, and LeDoux, 1997; Brioni, Nagahara, and McGaugh, 1989; Izquierdo, Quillfeldt, Zanatta, Quevedo, Schaeffer, Schmitz and Medina, 1997; Castellano, Brioni, Nagahara and McGaugh, 1989; Dickinson-Anson and McGaugh, 1997; Wilensky, Schafe, and LeDoux, 1999; Wilensky, Schafe, and LeDoux, 2000; Maren, Yap, and Goosens, 2001). For example, infusions of the gamma-aminobutyric acid ($GABA_A$) receptor agonist muscimol hydrobromide into the BLA of rats before Pavlovian fear conditioning has been shown to prevent any "savings" of auditory fear conditioning (Maren, Yap, and Goosens, 2001). Muscimol-infused rats in this study did not reacquire fear any faster than vehicle-infused and sensitized control rats after retraining sessions and their rate of reacquisition was no better than rats administered the vehicle solution (i.e. saline) which seems to indicate that few if any fear memories were established or saved when muscimol was active in the BLA during the original fear conditioning trials (Maren, et al., 2001). Thus, rats that received muscimol infusions prior to fear learning were unable to access a CS-UCS fear-memory trace and as a consequence were unable to exhibit elevated levels of reacquisition that would be indicative of fear-memory "savings"(Maren, et al., 2001). In a similar fashion, muscimol applied to the basolateral amygdaloid complex of rats was shown to impair both fear learning and fear

expression in a defensive freezing behavioural paradigm (Helmstetter and Bellgowan, 1994). More specifically, intra-basolateral amygdaloid infusion of a 0.5 μg dose of muscimol prior to contextual fear conditioning, before retention testing, or before both fear conditioning and final testing produced unmistakable deficits in the ability of rats to express freezing behaviour to contextual cues that had been explicitly paired with footshocks (Helmsteller and Bellgowan, 1994). Consistent with this result, a similarly designed study carried out by Muller, Corodimas, Fridel and LeDoux (1997) also demonstrated that activation of $GABA_A$ receptors in the basolateral amygdaloid nuclear region with muscimol blocked the acquisition and expression of both auditory and contextually conditioned fear responding in rats.

Furthermore, muscimol application to the central amygdaloid nucleus and dorsomedial aspects of the basolateral nucleus of the amygdala also blocked the acquisition and expression of fear-induced conditioned defeat in Syrian hamsters (Jasnow and Huhman, 2001). In this paradigm, hamsters that are exposed to a larger and more dominant male within the confines of the opponent's cage quickly develop or exhibit fear-motivated social defeat. As a consequence, socially defeated hamsters are usually unable to reverse their subordinate status and often display significantly lower levels of territorial aggression towards smaller and non-aggressive hamsters when they are returned to their home cage environment (Jasnow and Huhman, 2001). Intra-amygdalar infusion of muscimol (0.0, 4.4, or 8.8 nanomoles) either prior to social defeat training or just before final testing significantly attenuated this fear-motivated social defeat response in hamsters (Jasnow and Huhman, 2001). This data suggests that GABA receptor-mediated neurosynaptic transmission occurring primarily within the confines of the amygdala is likely a contributing factor that makes the acquisition and expression of this type of fear/stress-induced response possible (Jasnow and Huhman, 2001).

In addition to the reports on muscimol's influence on Pavlovian conditioned fear and stress-induced social responses, other experiments have shown that inhibitory avoidance retention and/or memory consolidation is severely impaired when this $GABA_A$ receptor agonist is introduced into the amygdala immediately after training (Brioni, Nagahara, and McGaugh, 1989; Izquierdo, Quillfeldt, Zanatta, Quevedo, Schaeffer, Schmitz and Medina, 1997; Castellano and McGaugh, 1990; Jerusalinsky, Quinfeldt, Waltz, Da Silva, Bueno e

Silva, Bianchin, Schmitz, Zannata, Ruschel, Paczko, Medina, and Izquierdo, 1994). Consistent with the theme that GABAergic receptor agonists interfere with fear-motivated learning, research has also demonstrated that systemic or intra-amygdaloid administration of the $GABA_B$ receptor agonist baclofen after inhibitory avoidance training disrupts the behavioural expression of this response during retention testing (Castellano, et al., 1989). In stark contrast, either post-training infusions into the amygdala or systemic administration of the $GABA_A$ receptor antagonist bicuculline have been shown to enhance inhibitory avoidance performance during retention testing, which seems to indicate that antagonism of GABA receptors play an active role in facilitating and modulating fear memory storage (Ammassari-Teule, Pavone, Castellano, and McGaugh, 1991; Brioni, Nagahara, and McGaugh, 1989; Castellano, et al., 1989).

In fact many of the research scientists who have conducted experiments in the inhibitory avoidance field tend to argue that post-training inactivation of basolateral amygdaloid neurons with muscimol and baclofen probably interferes with fear-motivated behaviours during retention testing by inhibiting amygdaloid processes that help modulate memory consolidation in other brain areas shortly after training is complete (Brioni, et al., 1989; McGaugh, et al., 1993; Izquierdo, et al., 1997). These researchers have collectively amassed some impressive evidence from the inhibitory avoidance paradigm that supports their claim that GABAergic amygdaloid processes modulate fear memory storage in other brain regions. However, there is ample research evidence that indicates that such an assertion may not be applicable to Pavlovian fear conditioning techniques that have manipulated GABAergic systems within the amygdala (Wilensky, Schafe and LeDoux, 1999; Maren, Yap and Goosens, 2001). Thus these studies have consistently shown that all the critical events required for fear learning and memory consolidation may be occurring within the confines of certain amygdaloid nuclei (Wilensky, Schafe and LeDoux, 1999; 2000; Maren, Yap and Goosens, 2001). For example, recent research by Wilensky and colleagues (2000) has clearly demonstrated that immediate post-training infusions of muscimol into the BLA had no detrimental impact on the expression of Pavlovian conditioned fear measured by defensive freezing behaviour, but did cause a significant dose-dependent impairment of inhibitory avoidance behaviour during retention testing carried out 48 hours later (Wilensky, et al., 2000). Furthermore, functional inactivation of

basolateral amygdaloid nuclei neurons with pre-training muscimol infusions dose-dependently blocked the acquisition of freezing behaviour to an auditory CS (Wilensky, et al., 2000). These findings are in agreement with earlier studies that have demonstrated that pre-treating amygdaloid neurons with muscimol generally blocks fear learning (Helmstetter and Bellgowan, 1993; Muller, et al., 1997; Jasnow and Huhman, 2001; Wilensky, Schafe and LeDoux, 1999) and prevents behavioural "savings" of auditory fear conditioning (Maren, Yap, and Goosens, 2001). The findings obtained by Wilensky and associates (2000) and various others (Helmstetter and Bellgowan, 1993; Muller, et al., 1997; Jasnow and Huhman, 2001; Wilensky, Schafe and LeDoux, 1999) make it logical to conclude that the amygdala plays a prominent role in acquiring CS-UCS associations during Pavlovian fear conditioning and most likely helps to modulate memory consolidation in remote brain regions that are exclusively devoted to inhibitory avoidance responding that relies more heavily on instrumental learning where shock delivery is contingent on the type of behavioural responses an animal performs (Wilensky, et al., 2000; McGaugh, et al., 1993; Castallano, et al., 1989; Brioni, et al., 1989; Izquierdo, et al., 1997). Thus, perhaps the most parsimonious explanation is that during Pavlovian fear conditioning the basolateral amygdaloid complex serves as a brain region where synaptic integrations, modifications, and biochemical events combine in such a way so as to not only acquire CS-UCS associations but also to permanently store and eventually retrieve fear memories that are stored within the amygdala itself. In contrast, during inhibitory avoidance learning specific nuclei in the amygdala may help to adjust the strength of fear memories stored in remote brain regions and during appropriate times these nuclei may recruit or retrieve these memories so that the amygdala can initiate adaptive and highly specialized responses that ensure that threatening or aversive environmental stimuli are avoided. In any event, the above discussions indicate that GABA receptors located on amygdaloid neurons play a key role in integrating synaptic signals and processing information that is vital to fear learning and expression.

6.7: The Role of the Mesoamygdaloid DA System and Amygdaloid DA Receptors in Pavlovian Fear Learning, Fear Retrieval and Fear Expression

Although numerous studies have examined the involvement of glutamate and GABAergic amygdaloid receptors in fear learning, memory consolidation, and fear expression, a growing body of research literature has begun to draw attention to the important role played by mesoamygdaloid DA neurodynamics in Pavlovian fear conditioning (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997; Waddington-Lamont and Kokkinidis, 1998; Nader and LeDoux, 1999; Guarraci, Frohardt and Kapp, 1999; Guarraci, and Kapp, 1999; Guarraci, Frohardt, Falls and Kapp, 2000; Greba and Kokkinidis, 2000; Greba, Gifkins and Kokkinidis, 2001; Gifkins, Greba and Kokkinidis, 2002). The dopaminergic system in the mammalian brain is divided into four functionally distinct neural pathways, these include; the nigrostriatal, tuberoinfundibular, mesocortical, and memolimbic DA pathways. In brief, the nigrostriatal DA pathway consists of DA neurons of the SN (A9) region and their efferent projections to the striatum (Swanson, 1982; Oades and Halliday, 1987; Kandel, 2000, Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Civelli, Bunzow, and Grady, 1993; Nolte, 1993; Vallone, Picetti, and Borrelli, 2000). The nigrostriatal pathway appears to play a critical role in triggering motor responses as a loss of the DA producing neurons in the A9 region and the subsequent degeneration of this pathway is one of the leading causes of Parkinson's disease (Kandel, 2000, Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Civelli, Bunzow, and Grady, 1993; Nolte, 1993; Vallone, et al., 2000). The tuberoinfundibular DA tract stems from the DA releasing neurons of the A12 and A14 regions near the third ventricle which contain various hypothalamic nuclei that make up what is commonly referred to as the tuberal hypothalamic region or the tuberoinfundibular-hypophyseal system (i.e. ventral medial nucleus, lateral nucleus, lateral tuberal nucleus, periventricular nucleus and the arcurate{infundibular}nucleus) (Björklund, Moore, Nobin, and Stenevi, 1973; Nolte, 1993; Demarest and Moore, 1979; Kandel, 2000; Civelli, et al., 1993; Moore and Lookingland, 1995; Vallone, et al., 2000). DA released by neurons of the A12 and A14 regions is routed into the portal bloodstream via fenestrated capillaries where it finally comes in contact with the pituitary gland, an area that reportedly expresses high levels of DA D_2 mRNA (Kandel, 2000, Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Civelli, et al., 1993; Nolte, 1993;

Giros, Sokoloff, Martres, Riou, Emorine and Schwartz, 1989; Monsma, McVittie, Gerfen, Mahan, and Sibley, 1989; Jackson and Weslind-Danielsson, 1994). In the pituitary gland DA released by the A12 and A14 hypothalamic neurons behaves like a regulating hormone rather than a neurotransmitter in that it serves to exert control on the levels of prolactin released from the pituitary gland (Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Moore and Lookingland, 1995). It has been suggested that DA released from the arcurate nucleus of the hypothalamus binds to DA D_2-like receptors found on lactotrophs and helps to regulate prolactin release (Jackson and Westlind-Danielsson, 1994; Moore and Lookingland, 1995). In doing so, DA neurons in the hypothalamus (i.e. A12 and A14 groups) can interact with elements of the neuroendocrine system and play an important role in influencing fertility and lactation in many mammalian species (Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Moore and Lookingland, 1995; Vallone, et al., 2000).

Both the mesocortical and mesolimbic DA pathways originate from DA neurons found in the A10 group of the VTA (Swanson, 1982; Oades and Halliday, 1987; Saper, 2000 in Kandel, Schwartz and Jessell, 2000; Vallone, et al., 2000). In the mesocortical dopaminergic system ventral tegmental neurons of the A10 group innervate several frontal and temporal neocortical areas that are thought to participate in higher order functioning (i.e. social behaviour, attention, motivation and planning) these include; the medial prefrontal cortex, anterior cingulate cortex, pyriform cortex and entorhinal cortex (Berger, Thierry, Tassin and Moyne, 1976; Emson, and Koob, 1978; Swanson, 1982; Kandel, 2000, Saper, 2000 in Kandel, Schwartz and Jessell, 2000). Efferents from ventral tegmental DA neurons belonging to the mesolimbic dopaminergic system take a different route and end up making robust connections with several limbic brain regions that are believed to participate in the production of autonomic and behavioural responses commonly associated with highly charged emotional states (e.g. fear, anxiety, and defensive aggression) (Swanson, 1982; Oades and Halliday, 1987; Phillipson, 1979; Brinley-Reed and McDonald, 1999; Kandel, 2000, Saper, 2000 in Kandel, Schwartz and Jessell, 2000). The limbic regions receiving innervation from the ventral tegmental DA neurons include areas such as the nucleus accumbens, ventral striatum, hippocampus, lateral septum, BNST, and of

course the amygdala (Fallon, Koziell and Moore, 1978; Phillipson, 1979; Swanson, 1982; Loughlin and Fallon, 1983; Brinley-Reed and McDonald, 1999; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). Other regions targeted by the VTA mesolimbic DA neurons include portions of the anterior cingulate cortex and parts of the entorhinal cortex, which suggests that there is probably a significant degree of overlap between the mesocortical and mesolimbic dopaminergic systems (Swanson, 1982; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). For this reason DA fibres emanating out of the A10 cell group and targeting limbic and cortical regions have sometimes been lumped together to form a larger category called the mesocorticolimbic DA system (Civelli, et al., 1993). Because numerous research studies have demonstrated that mesolimbic and specifically the mesoamygdaloid DA system are involved in the learning and expression of conditioned fear and several stress related autonomic and behavioural responses (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997; Waddington-Lamont and Kokkinidis, 1998; Nader and LeDoux, 1999; Guarraci, et al., 1999; Guarraci, and Kapp, 1999; Guarraci, et al., 2000; Greba and Kokkinidis, 2000; Greba, et al., 2001; Gifkins, Greba and Kokkinidis, 2002; Inglis, and Moghaddam, 1999; Herman, et al., 1982; Coco, et al., 1992), most dialogue in the sections that follow will be based upon experimental investigations that have manipulated this particular catecholaminergic system. However, it is first necessary to briefly examine how interest in DA and dopaminergic systems as it pertains to emotionality and fear began and then progressively evolved.

6.71: Neuroleptic Drugs Alleviate Paranoid Psychosis and the Dopamine System in the CNS is Mapped

Ever since the discovery that neuroleptic drugs were effective pharmacological agents that could be used to treat paranoid psychosis and ameliorate many of the symptoms commonly associated with schizophrenia, efforts within the scientific community have been directed towards determining how DA-mediated neural events were involved in emotional pathology (i.e. paranoia and schizophrenia) and fear learning and fear expression. In the early 1950's and during the twenty years that followed, it became quite obvious that chlorpromazine (a typical neuroleptic belonging to the phenothiazine class) and haloperidol (a butyrophenone derivative) were effective in abolishing most psychotic

symptoms of schizophrenia such as hallucinations, delusional though patterns, fragmented cognition as well as paranoid ideations, fearfulness and anxiety-like behaviours (Carlsson, 1974; Snyder, Banerjee, Yamamura, and Green, 1974; Carlsson, 1977; Kandel, 2000; Julien, 1996). As medical research progressed new and different classes of antipsychotic drugs were synthesised and these became known as the atypical antipsychotics (i.e. clozapine, olanzapine and risperidone) (Carlsson, 1974; Carlsson, 1977; Snyder, et al., 1974; Kandel, 2000; Julien, 1996). These new compounds were employed not only as a means of alleviating the cognitive and emotional pathologies associated with schizophrenia but were also used in an effort to reduce the undesirable extrapyramidal side effects caused by the typical class of antipsychotic drugs (Kandel, 2000; Julien, 1996).

A number of significant early scientific advancements paved the way for our present day understanding of the role of mesoamygdaloid dopaminergic systems in fear acquisition and expression. These included, the detection of DA neurons in the VTA and SN by Dahlstrom and Fuxe (1964), the discovery that antipsychotic drugs may work by blocking DA receptors (Carlsson, 1974; Carlsson, 1977; also see Kandel, 2000 in Kandel, Schwartz and Jessell, 2000), the detailed description of the projection fields of DA neurons (Swanson, 1982; Loughlin and Fallon, 1983; Oades and Halliday, 1987; Brinley-Reed and McDonald, 1999) and finally the subsequent experimental cloning, autoradiographic binding studies and in situ hybridization experiments that have provided definitive evidence that DA D_1 and D_2 receptors exist throughout various regions in the mammalian brain (Dawson, Barone, Sidhu, Wamsley, and Chase, 1988; Boyson, McGonigle, and Molinoff, 1986; Wamsley, Gehlert, Filloux and Dawson, 1989; Meador-Woodruff, Mansour, Bunzow, Hubert, Van Tol, Watson, and Civelli, 1989; Meador-Woodruff, Mansour, Healy, Kuehn, Zhou, Bunzow, Akil, Civelli, and Watson, 1991; Bunzow, Van Tol, Grandy, Albert, Salon, Christie, Machida, Neve, and Civelli, 1988; Zhou, Grandy, Thambi, Kushner, Van, Cone, Pribnow, Salon, Bunzow and Civelli, 1990; Monsma, Mahan, McVittie, Gerfen, and Sibley, 1990; Sunahara, Niznik, Weiner, Stormann, Brann, Kennedy, Gelernter Rozmahel, Yang, Israel, Seeman, and O'Dowd, 1990; for a review also see Jackson and Westlind-Danielsson, 1994; Civelli, Bunzow, and Grandy, 1993).

The first scientific effort to shed light on the distribution and location of DA neurons was made by Dahlstrom and Fuxe (1964). By implementing histofluorescence techniques,

these researchers were able to demonstrate the existence of the A9 and A10 DA cell groups located within the confines of the SN and VTA respectively (Dahlstrom and Fuxe, 1964). Ensuing anatomical, immunofluoresent, autoradiographic and tracing experiments helped to provide a comprehensive depiction of the terminal projection fields of the DA neurons of the VTA (A10) and SN (A9) (Thierry, Blanc, Sobel, Stinus, and Glowinski, 1973; Fuxe, Hökfelt, Johansson, Jonsson, Lidbrink, and Ljungdahl, 1974; Lindvall, 1975; Lindvall and Björklund, 1974; Lindvall and Björklund, and Divac, 1977; 1978; Lindvall, Björklund, Moore, and Stenivi, 1974; Berger, Tassin, Blanc, Moyne, and Thierry, 1974; Berger, Thierry, Tassin and Moyne, 1976; Carter and Fibiger, 1977; Emson, and Koob, 1978; Fallon and Moore, 1978a,b; Fallon, Koziell and Moore, 1978; Beckstead, Domesick and Nauta, 1979; Simon, Le Moal, and Calas, 1979; Swanson, 1982; Loughlin and Fallon, 1983; Oades and Halliday, 1987). These studies have ascertained that ventral tegmental DA neurons send ascending projections mostly via the medial forebrain bundle to various limbic and cortical brain areas (Swanson, 1982). For example, as mentioned earlier, ventral tegmental DA neurons of the A10 group innervate the nucleus accumbens and the ventromedial caudate-putamen (i.e. striatum) (Beckstead, et al, 1979; Swanson, 1982; Linvall and Björklund, 1979). Swanson (1982) observed that of the ventral tegmental cells that projected to the nucleus accumbens and striatum approximately eight-five percent responded to the bovine adrenal tyrosine hydroxylase antiserum during staining, thus indicating that a vast majority of ventral tegmental projection neurons were indeed dopaminergic. The ventral tegmental neurons also supply DA input to the hippocampus, ventral pallidum, BNST and lateral septal nucleus (Herve, et al., 1979; Swanson, 1982; Oades and Halliday, 1987; Domesick, 1988). In addition to this, efferents emanating from ventral tegmental DA neurons are distributed throughout much of the medial prefrontal cortex and various cortico-limbic regions such as the entorhinal, piriform and cingulate cortices (Thierry, Blanc, Sorbel, Stinus and Glowinski, 1973; Fuxe, Hökfelt, Johansson, Johsson, Lidbrink and Ljungdahl, 1974; Lindvall, et al., 1974; Swanson, 1982; Lindvall and Björklund, 1987).

Perhaps the most important finding made by several research groups has been the discovery that DA neurons of the VTA provide considerable dopaminergic input to several amygdaloid nuclei that are evidently involved in conditioned fear learning and expression

(Ben-Ari, Zigmond, and Moore, 1975; Fallon, et al., 1978; Phillipson, 1979; Swanson, 1982; Oades and Halliday, 1987; Loughlin and Fallon, 1983; Asan, 1997; Brinley-Reed and McDonald, 1999; Davis, 1992a; Fendt and Fanselow, 1999; Maren, 1999; Nader and LeDoux, 1999; Waddington-Lamont and Kokkinidis, 1998; LeDoux, 2000; Walker and Davis, 2000; Greba and Kokkinidis, 2000). Specifically, VTA DA neurons innervate cells situated in the central, medial, cortical, basal, and basolateral amygdaloid nuclear regions (Phillipson, 1979; Swanson, 1982; Loughlin and Fallon, 1983; Oades and Halliday, 1987; Brinley-Reed and McDonald, 1999) and because some of these amygdaloid nuclei have been shown to contain substantial populations of DA D_1 and D_2 receptor subtypes (Bouthenet, Martres, Sales, and Schwartz, 1987; Wamsley, et al., 1989; Dawson, et al., 1988; Boyson, et al., 1986; Meador-Woodruff, et al., 1989; Meador-Woodruff, Mansour, Healy, Kuehn, Zhou, Bunzow, Akil, Civelli, and Watson, 1991) it is highly probable that mesoamygdaloid DA and DA receptor-mediated events could play a major role in orchestrating neurobiochemical events that enhance fear-motivated learning and fear expression.

6.72: DA Neurons and DA Receptors: Distribution, Location and Characteristic Features, and Biochemistry

DA receptors are widely distributed throughout the mammalian brain and they can be divided into several distinct subtypes, these include D_1, D_2, D_3, D_4, and D_5 subtypes (Civelli, Bunzow, and Grandy, 1993; Jackson and Westlind-Danielsson, 1994; Seeman, 1995; Mansour and Watson, 1995; Missale, Nash, Robinson Jaber, and Caron, 1998; Vallone, et al., 2000; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). Although cloning experiments have added three additional subtypes to the previously identified D_1 and D_2 receptors, it is generally recognized that D_3, D_4 and D_5 subclasses can be designated as belonging to either the D_1-like or the D_2-like DA receptor category (Giros, Martres, Sokoloff, and Schwartz, 1990; Sokoloff, Giros, Martres, Bouthenet, and Schwartz, 1990; Van Tol, Bunzow, Guan, Sunahara, Seeman, Niznik, and Civelli, 1991; Jackson and Westlind-Danielsson, 1994; Civelli, et al., 1993; Seeman, 1995; Mansour and Watson, 1995 Missale, et al., 1998; Vallone, et al., 2000). For example, the D_5 receptor subtype is included in the D_1-like family since it has many biochemical, molecular, and genetic traits

of the D_1 receptor (Civelli, Bunzow, and Grandy, 1993; 1990; Van Tol, Bunzow, Guan, Sunahara, Seeman, Niznik, and Civelli, 1991; Jackson and Westlind-Danielsson, 1994; Mansour and Watson, 1995; Vallone, et al., 2000). In contrast, the D_3 and D_4 receptor subtypes are subsumed in the D_2-like family as they share many characteristics of the D_2 DA receptor (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Mansour and Watson, 1995; Missale, et al., 1998; Vallone, et al., 2000). The ability to dissociate between D_1 and D_2 DA receptors and organize the other receptors subtypes into either the D_1-like or D_2-like category is largely based upon the way in which various dopaminergic agonists and antagonists were observed to interact with the cyclic adenosine monophosphate (cAMP) second message generating enzyme adenylyl cyclase (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994). The application of DA to neuronal tissue for example, typically enhances both adenylyl cyclase and cAMP production and this effect can be blocked by the application of DA D_2 receptor antagonists (see Jackson and Westlind-Danielsson, 1994). Thus, adenylyl cyclase activity appears to be inhibited by D_2 receptors. As a matter of fact, there is considerable consensus in the research literature that indicates that DA D_1 receptors interact with G_s-protein molecules to activate adenylyl cyclase and increase levels of intracellular cAMP, whereas the D_2 receptor forms linkages with the G_i and G_o G-protein compounds to inhibit the production of cAMP (Ongini, and Longo, 1989; Blackburn, Pfasu and Phillips, 1992; Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Vallone, et al., 2000; Gingrich and Caron, 1993). It is also important to note that the scheme used to categorize DA receptors has been aided by examining the binding affinities of known dopaminergic agonist or antagonist drugs with the D_1 and D_2 receptors (Jackson and Westlind-Danielsson, 1994). This was done in order to find out whether the newly cloned DA receptors (D_3, D_4 and D_5) matched the pharmacological profiles that had been established for D_1 and D_2 receptors.

6.73: DA Receptors and the Molecular Structure of DA Receptors

All DA receptors belong to the G-protein-coupled family of receptors and as a result they all possess the seven hydrophobic α-helical transmembrane spanning domains that is a prominent feature found in all G-protein coupled receptor types (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Missale, et al., 1998; Vallone, et al., 2000;

Siegelbaum, Schwartz, and Kandel, 2000; Kandel, 2000). Each transmembrane spanning region is embedded in the plasma membrane and these are numbered TM1 to TM7. DA receptors also contain three cytoplasmic loops (CPL1, CPL2, and CPL3) and three extracellular loops (O1, O2, and O3) with a carboxy (COOH) C- terminus located in the intracellular cytoplasm and an amino (NH2) terminus situated in the extracellular matrix (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Missale, et al., 1998; Vallone, et al., 2000; Siegelbaum, Schwartz, and Kandel, 2000; Kandel, 2000). Although D_1-like and D_2-like DA receptors have some very basic things in common, it is valuable to point out that there are certain fundamental pharmacological, structural and biomolecular differences that set the two family groups apart. These differences are particularly important in that any slight variation in any one of the variables could influence the binding affinity of DA agonists and antagonists and affect how particular DA receptor subtypes stimulate or inhibit adenylyl cyclase activity and the subsequent cAMP-PKA-ERK-MAPK signalling pathway that is necessary for generating LTP induction and memory formation during Pavlovian fear conditioning (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Vallone, et al., 2000; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000; Schafe, et al., 2001). One prominent feature that distinguishes DA D_2-like receptors from the D_1-like receptors (i.e. D_1 and D_5) is all D_2-like receptors (D_2, D_3 and D_4) have a very long amino acid chain on CPL3, whereas the D_1-like receptors' third cytoplasmic loop is much shorter in length (Jackson and Westlind-Danielsson, 1994). Another unique difference is that D_2-like receptors have a short carboxy (COOH) C-terminal while their D_1-like relatives have a C-terminal tail that has been estimated to be approximately seven times longer (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994). It has been suggested that the length of CPL3 found in the D_2 receptors is a typical structural attribute of D_2-like receptors that inhibit adenylyl cyclase by coupling to specific G-proteins (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Gingrich, and Caron, 1993; Missale, et al., 1998; Vallone, et al., 2000). Another glaring structural difference between D_1-like and D_2-like receptors is the fact that D_1-like receptors do not contain introns in protein/gene coding regions (Civelli, et al., 1993; Jackson and Westlind-Danielsson, 1994; Gingrich and Caron, 1993; Missale, et al., 1998; Vallone, et al., 2000). In stark contrast, D_2-like dopaminergic receptors (i.e. D_2, D_3, and D_4) do contain introns in their protein/gene coding regions (Civelli, et al., 1993;

Jackson and Westlind-Danielsson, 1994; Gingrich and Caron, 1993; Missale, et al., 1998; Vallone, et al., 2000). The presence of introns make it possible to generate different D_2-like variants (i.e. D_2 long, and D_2 short) from the original D_2 receptor through a process called alternative splicing (Civelli, et al., 1993; Gingrich, and Caron, 1993; Jackson and Westlind-Danielsson, 1994; Mansour and Watson, 1995; Vallone, et al., 2000). Similar D_3-long and D_3-short variants can also be produced from the D_3 receptor by splicing out an amino acid peptide in the third cytoplasmic loop (Fishburn, Belleli, David, Carmon, and Fuchs, 1993 in Seeman, 1995). Hence, the existence of numerous introns in D_2, D_3, and D_4 receptor genes makes it possible to produce a number of variants of these receptors (Mansour and Watson, 1995; Jackson and Westlind-Danielsson, 1994).

Generally speaking, the DNA of each gene that takes part in the encoding of protein molecules required for cellular transformations usually consists of numerous segments referred to as exons (Gilliam, Kandel, and Jessell, 2000 in Kandel, Schwartz, and Jessell, 2000). Exons are normally responsible for encoding certain parts of a protein molecule. In most instances, the exons (i.e. coding segment on each DNA) are periodically interrupted by introns which are the non-coding segments that are interspersed amongst the exons (Gilliam, Kandel, and Jessell, 2000 in Kandel, Schwartz, and Jessell, 2000). The presence or absence of introns or exons has been quite valuable in that it makes it possible to identify and classify DA receptors and this is true even when receptors belong to the same family class. For example, the gene belonging to the D_2 receptor is made up of eight exons and seven of these are used for coding, whereas the D4 receptor contains only five exons all of which are coding (Civelli, et al., 1993; Gingrich and Caron, 1993; Jackson and Westlind-Danielsson, 1994; Vallone, et al., 2000). Moreover, the genes of the D_1-like receptors (i.e. D_1 and D_5) possess no introns whatsoever while all receptors belonging to the D_2-like family (i.e. D_2, D_3 and D_4) do (Civelli, et al., 1993; Gingrich and Caron, 1993; Jackson and Westlind-Danielsson, 1994; Vallone, 2000).

6.74: Location of DA Receptors in the Brain

There is a wealth of research evidence gathered from a variety of different scientific laboratories that indicates that DA receptors can be found throughout many of the nuclear cell groupings of the mesolimbic, mesocortical and nigrostriatal regions in the rat central

nervous system (Boyson, et al., 1986; Dawson, et al., 1988; Wamsley et al., 1989; Bouthenet, et al., 1987; Charuchinda, et al., 1987; DuBois, et al., 1986; Martres, et al., 1985; Meador-Woodruff, et al., 1989; 1991; Jackson and Westlind-Danielsson, 1994; Mansour and Watson, 1995). The vast majority of this research suggests that D_1 and D_2 mRNA is found throughout several neuronal populations and brain structures that belong to one of the dopaminergic systems (Meador-Woodruff, et al., 1989; 1991; Weiner and Braun, 1989; Dearry, Grinrich Falardeau, Fremeau, Bates and Caron, 1990; Monsma, et al., 1990; Sunahara, et al., 1990; Mansour and Watson, 1995). For example, both D_1 and D_2 mRNA is expressed in the septum, hypothalamus, caudate-putamen, nucleus accumbens and various cortical regions (e.g. medial prefrontal cortex, entorhinal cortex and cingulate cortex) (Meador-Woodruff, et al., 1989; 1991; Mansour and Watson, 1995). In addition, DA neurons in the VTA and SN appear to contain high numbers of D_2 and perhaps D_3 DA receptors especially since D_2 mRNA is expressed in these two regions and the binding affinity for D_2 antagonists and agonists is fairly high in these two mid-brain areas (Bouthenet, Martres, Sales, and Schwartz, 1987; Charuchinda, Suparvilai, Karobath, and Palacios, 1987; DuBios, Savasta, Curet, Scatton, 1986; Martres, Bouthenet, Sales, Sokoloff and Schwartz, 1985; Wamsley, et al., 1989; Meador-Woodruff, et al., 1991; Mansour and Watson, 1995). For example, Wamsley and associates (1989) along with various others (Bouthenet, et al., 1987; Charuchinda, et al., 1987) who examined D_2 receptor binding levels using autoradiographic techniques found that D_2 receptor density in the VTA was quite substantial but somewhat lower than that observed in the SN. Consistent with these research reports, Meador-Woodruff and his research group (1989; 1991) found high levels of D_2 receptor mRNA in the VTA and SN in two separate studies that employed in situ hybridization methods. Both of these experiments reported that D_2 mRNA concentrations in the VTA and SN were far higher than D_2 mRNA levels found in the nucleus accumbens and caudate-putamen (Meador-Woodruff, et al., 1989; 1991). This seems to indicate that a large population of D_2 receptors is likely located on the DA releasing neurons of the VTA and laterally situated SN (Mansour and Watson, 1995).

It is hypothesised that D_2 (and quite possibly D_3) DA receptors in these two regions are located on the terminal points of presynaptic neurons and therefore behave as autoreceptors that function to inhibit the frequency and rate of firing of DA neurons (White and Wang,

1984a; White and Wang, 1984b; White and Wang, 1984c; Jackson and Westlind-Danielsson, 1994; Kalivas, 1993). This notion is supported by electrophysiological studies that have demonstrated that iontophoretic or systemic application of the D_2/D_3 receptor agonist quinpirole or amphetamine reduces both the burst firing and the overall firing rate of DA neurons in the VTA and SN (Bunny, Walters, Roth, and Aghajanian, 1973; Akaoka, Charléty, Saunier, Buda, and Chouvet, 1992; White and Wang, 1984a; White and Wang, 1984b; Ackerman, Johansen, Clark, and White, 1983). Consistent with this train of research, DA D_2 receptor agonists reduce dopaminergic metabolites HVA (homovanillic acid) and DOPAC (3, 4,-dihydroxyphenylacetic acid) in terminal regions innervated by the VTA and SN (Bull, Bakhtiar, and Sheehan, 1991; Jackson and Westlind-Danielsson, 1994) whereas, DA D_2 receptor antagonists increase the levels of these dopaminergic metabolites in terminal areas and generally cause an enhancement of DA cell firing after acute administration (see Jackson and Westlind-Danielsson, 1994; Bunney, et al., 1973). For instance, microinfusion of the DA D_2 receptor antagonist (-) sulpiride into the mid-brain tegmental nuclei disinhibits neurons residing in this region and as a result increases cellular firing rates and extracellular DA levels in regions of the limbic striatum (Pirot, Godbout, Mantz, Tassin, Glowinski, and Thierry, 1992; Westerink, Kwint, and de Vries, 1996). Similarly, administration of neuroleptic compounds such as haloperidol or anti-emetic agents such as metaclopramide, both of which antagonize D_2 DA receptors, have been shown to elevate the quantity of DA metabolites found in terminal regions innervated by DA neurons of the mid-brain VTA and SN (Boyar and Altar, 1987).

This seems to indicate that the regulation of DA cell activity and the release of dopaminergic metabolites may fall under the control of both terminal and somatodendritic D_2 DA receptors (Jackson and Westlind-Danielsson, 1994; Kalivas, 1993; Roth and Elsworth, 1995). Furthermore, the suppression of neuronal activity in the VTA and SN after D_2 agonist administration can be reversed by neuroleptic drugs that act primarily on postsynaptic D_2 DA receptors in terminal areas and on D_2 autoreceptors (Bunney, et al., 1973; Jackson and Westlind-Danielsson, 1994; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). For example, early work conducted by Bunney and colleagues (1973) clearly demonstrated that several antipsychotic drugs (e.g. chloropromazine, haloperidol, trifluoperazine, thioridazine, periphenazine and fluphenazine) rapidly reversed the

inhibitory effects on VTA and SN neurons caused by intravenous d-amphetamine administration and actually increased DA cell activity above baseline levels in these two brain areas (Bunney, et al., 1973). Because D_2 receptor antagonists increase DA turnover in terminal regions and reverse many of the effects produced by D_2 receptor agonists (i.e. quinpirole) and DA reuptake inhibitors (i.e. amphetamine), it is plausible that blockade of postsynaptic D_2 DA receptors and D_2 autoreceptors with neuroleptic drugs or selective D_2 antagonists might initially lead to a quick feedback-mediated compensatory increase in dopaminergic cellular activity and a subsequent release of DA into terminal areas in the short term so as to deal with any perceived loss of DA tone (Bunney, et al., 1973; Jackson and Westlind-Danielsson, 1994; Kandel, 2000 in Kandel, Schwartz and Jessell, 2000). However, with the passage of time antipsychotic medication or selective D_2 receptor antagonists may eventually reduce the activity level of mesolimbic DA neurons and thus lower DA turnover in terminal areas that receive dopaminergic input from the VTA and SN. As a matter of fact, Skarsfeldt (1988a,b; 1992) used both typical and atypical antipsychotic drugs administered in sub-chronic fashion to prove that mesolimbic and nigro-striatal dopaminergic neuronal activity could be suppressed after prolonged treatment with these pharmacological agents. Across a series of experiments Skarsfeldt (1988a,b; 1992) found that atypical antipsychotic drugs (clozapine, remoxipride and sertindole) were very effective in inhibiting the activity of mesolimbic DA neurons after a twenty-one day treatment period. Taken together, the above discussion suggests that mesolimbic dopaminergic receptors are critically involved in regulating midbrain DA neuronal activity levels and DA metabolism, and as a consequence, they are probably the foremost neural substrates that contribute to the antipsychotic effects produced by neuroleptic drugs (for a review see Roth and Elsworth, 1995).

6.75: Dopamine Receptor Subtypes are Located in the Amygdala

Perhaps one of the most important points that must be stressed is that several lines of research indicate that D_1 and D_2 receptors are located in the amygdala and the amygdala is a limbic area that is well known to be involved in fear learning and expression (Meador-Woodruff, et al., 1989; 1991; Boyson, et al., 1986; Bouthenet, et al., 1987; Dawson, et al., 1988; Wamsley, et al., 1989; Scibilia, Lachowicz and Kilts, 1992; Mansour and Watson,

1995; Fuxe, Jacobsen, Höistad, Tinner, Jansson, Staines and Agnati, 2003; Davis, 1992a; 2000; LeDoux, 2000; Fendt and Fanselow, 1999; Maren, 1999). It fact autoradiographic and in situ hybridization experiments have determined that D_1 and D_2 receptors are located in the central nucleus of the amygdala, the lateral capsular subdivision of the central nucleus of the amygdala, the cortical and medial nuclei of the amygdala and the basolateral amygdaloid complex (Boyson, et al., 1986; Bouthenet, et al., 1987; Dawson, et al., 1988; Meador-Woodruff, et al., 1989; Wamsley, et al., 1989; Meador-Woodruff, et al., 1991; Scibilia, et al., 1992). Specifically, Boyson and colleagues (1986) estimated that in the basolateral and medial amygdaloid nuclei of the rat the ratio of D_1 to D_2 receptors was approximately 8:1 which indicates that fewer D_2 receptors are present in these particular amygdaloid nuclei. With regard to the central nucleus of the amygdala, Boyson et al., (1986) reported a 2:1 ratio of D_1 to D_2 DA receptors. Consistent with this report, more recent work has demonstrated that numerous DA D_2 receptors are located in the lateral subdivision of the central amygdaloid nucleus (Scibilia, et al., 1992). It is worth mentioning that even larger quantities of D_2 receptors were shown to be located in the lateral capsular subdivision of the central amygdaloid nucleus bordering the basolateral amygdaloid complex and the AStr laterally (Scibilia, et al., 1992). Overall these findings are generally consistent with reports that have also found more D_1 and less D_2 receptors in the amygdala (Wamsley, et al., 1989).

Despite this finding, it should also be noted that DA D_3 and D_4 receptors are present in the amygdala as well (see Jackson and Westlind-Danielsson, 1994; Coco and Kilts in Freedman and Cassell, 1994) and the presence of D_1, D_2 and D_3 receptor subtypes is particularly important as tyrosine hydroxylase immunoreactive fibres are found scattered throughout the basolateral amygdaloid nucleus, the lateral and medial divisions of the central amygdaloid nucleus, and within the amygdalostriatal transition zone (Fallon and Ciofi, 1992; Fuxe, Cintra, Agnati, Häfstrand and Goldstein, 1988; Gustafson and Greengard, 1990; Honkaniemi, 1992; Freedman and Cassell, 1994). This suggests that neurons within various amygdaloid regions not only contain DA receptors but are also the recipients of DA-mediated synaptic signals emanating from mid-brain DA neurons of the VTA (Oades and Halliday, 1987; Swanson, 1982; Loughlin and Fallon, 1983; Revay, Vaughan, Grant, and Kuhar, 1996; Asan, 1997; Brinley-Reed and McDonald, 1999). Given

this arrangement, it is highly probable that the mesoamygdaloid DA system plays a critical part in both conditioned fear learning and expression, and in the aetiology of paranoid schizophrenia and other similarly related disorders of thought, mood, and emotion.

6.76: The Mesolimbic DA System, Mesoamygdaloid DA Receptors and Conditioned Fear Learning and Expression

Over the past decade or so results obtained from a substantial number of studies have been used to explicate the role of the mesolimbic DA system in fear learning and expression (Borowski and Kokkinidis, 1996; Nader and LeDoux, 1999a,b; Munro and Kokkinidis, 1997; Borowski and Kokkinidis, 1998; Waddington-Lamont and Kokkinidis, 1998; Guarraci and Kapp, 1999; Guarraci, et al., 1999; 2000; Greba and Kokkinidis, 2000; Gelowitz and Kokkinidis, 1999; Gifkins, et al., 2002; Bissiere, Humeau, and Lüthi, 2003). On the basis of this work a consensus has emerged that the VTA DA neurons and efferent pathways to the amygdala in combination with D_1 and D_2 amygdaloid receptors form an important part of the neuroanatomical and biochemical synaptic circuitry that is essential for conditioned fear learning and expression. The scientific journey involved in making this discovery began approximately five decades ago. The first hint that DA receptors play an important role in conditioned fear stems from experimental efforts that employed conditioned avoidance responding to assess fear-motivated learning.

This line of research had its genesis in the 1950s and early 1960 around the time when antipsychotic drugs had been implemented as a pharmacological intervention for the treatment of schizophrenia (Blackburn, Pfaus, and Phillips, 1992). During this particular period several research groups reported that chlorpromazine administration resulted in marked impairments in active avoidance behaviour in laboratory animals (Ader and Clink, 1957; Courvoisier, 1956; Miller, Murphey and Mirsky, 1957; Posluns, 1962; Cook and Catania, 1964). It is undeniable that a substantial number of research reports and literature reviews over the years have drawn attention to the fact that a wide range of D_1 and D_2 dopaminergic receptor antagonists have the capacity to disrupt the acquisition of conditioned avoidance responding when they are administered either systemically or directly into discrete limbic brain nuclei (Davidson and Weildey, 1976; Arnt, 1982; Blackburn and Phillips, 1989; Ader and Clink, 1957; Posluns, 1962; Cook and Catania,

1964; Couvoisier, 1956; Miller, Murphey, and Mirsky, 1957; Niemegeers, Verbruggen and Janssen, 1969a,b; Iorio, Barnett, Leitz, Houser, and Korduba, 1983; Sanger, 1985; Sanger, 1987; Ichihara, Nabeshima and Kameyama, 1988; White, Ciancone, Haracz and Rebec, 1991; White and Rebec, 1994; Petty and Sacquitne, 1982; Petty, Mott, and Sherman, 1984; Blackburn, Pfaus, and Phillips, 1992; Salamone, 1994; Josselyn, Miller, and Beninger, 1997). For example, research by Arnt (1982) demonstrated that all classes of neuroleptic drugs (e.g. phenothiazines, thioxanthenes, butyrophenones, benzamides, phenylindanes, and atypical antipsychotics) inhibited conditioned avoidance responding in rats when given systemically. In particular, the atypical neuroleptic drug clozapine produced a considerable inhibition of conditioned avoidance responding without causing catalepsy (Arnt, 1982). More importantly, intra-amygdala infusion of DA receptor antagonists have been shown to cause significant delays in the ability of laboratory animals to acquire one-way avoidance responding (Sherman, et al., 1982; Petty, et al., 1984), suggesting that the detrimental effects on avoidance responding produced by systemically administered dopaminergic antagonists may be attributed to processes taking place within the amygdala.

Consistent with this possibility is the fact that 6 hydroxy-dopamine (6-OHDA) lesions of the amygdala have been shown to produce profound impairments in the acquisition of a two-way active avoidance response (Ashford and Jones, 1976). In this connection, it is noteworthy that 6-OHDA lesions of the VTA prevents rats from acquiring a two-way conditioned avoidance response (Oades, Rivet, Taghzouti, Kharouby, Simon, and Le Moal, 1987) and microinfusion of sulpiride into the nucleus accumbens blocks active avoidance behaviour (Wadenberg, Ericson, Magnusson and Ahlenius, 1990). These results are not surprising given the fact that the VTA contains numerous DA neurons and provides the amygdala and nucleus accumbens with a rich dopaminergic input (Oades and Halliday, 1987; Swanson, 1982; Loughlin and Fallon, 1983; Brinley-Reed and McDonald, 1999). Thus, it is conceivable that a dopaminergic circuit consisting of the VTA, amygdala and nucleus accumbens may be required for acquiring and generating fear-motivated responses such as conditioned active avoidance behaviour (Salamone, 1994; Blackburn, et al., 1992).

Although considerable attention has focused on the involvement of dopaminergic systems in conditioned avoidance responding, there is strong evidence that mesoamygdaloid DA systems may be essential for the acquisition and expression of

Pavlovian fear conditioning. As a matter of fact, scientific evidence has shown that neuroleptic drugs that antagonize DA D_2 receptors and reduce the emotional pathology associated with schizophrenia are relatively effective in blocking or dampening the expression of FPS triggered by a CS that was previously paired with an aversive event (Snyder, et al., 1974; Carlsson, 1977; Davis, et al., 1993; Hijzen, Houtzanger, Joordens, Oliver and Slagen, 1995). For example, systemic administration of either the D_1 DA receptor antagonist SCH 23390 or the D_2 DA receptor antagonist raclopride L-tartrate has been shown to reduce or block FPS in rats (Davis, et al., 1993; Greba and Kokkinidis, 2000). In this connection it is noteworthy that cocaine and d-amphetamine which ostensibly augment DA activity in the CNS, also increase paranoid ideations and anxiety in humans, exacerbate the positive symptoms of schizophrenia, and sensitise FPS responding in rats (Anthony, Tein, and Petronis, 1989; Aronson and Craig, 1986; Bell, 1973; Griffith, Cavanaugh, Held, and Oates, 1970; Sherer, 1988; Angrist, Rostrosen and Gershon, 1980; Borowski and Kokkinidis, 1994; Willick and Kokkinidis, 1995).

In addition, systemic administration of quinpirole (a D_2 DA receptor agonist that acts on D_2 autoreceptors to inhibit VTA DA neurons and DA release in terminal limbic regions) prior to a series of second order fear conditioning trials has been shown to dose-dependently block the acquisition of second order fear conditioning in a defensive freezing paradigm (Nader and LeDoux, 1999a; Lacey, Mercuri, and North, 1987; White and Wang, 1984). The level of defensive freezing exhibited by quinpirole-treated rats during exposure to the secondary conditioned stimulus (CS2) was significantly lower than saline-treated rats and this effect could be reversed by pretreating rats with the DA antagonist α-flupentixol (0.3 mg/kg) (Nader and LeDoux, 1999a). Furthermore, intraperitoneal injections of cocaine (40.0 mg/kg), d-amphetamine (5.0 mg/kg) or the specific D_1 receptor agonist SKF 38393 (5.0mg/kg) blocked the extinction of FPS in rats presumably by sensitising or activating mesoamygdaloid DA systems during extinction training (Willick and Kokkinidis, 1995; Borowski and Kokkinidis, 1998). It is also very important to note that cocaine and SKF 38393 administered to fear-extinguished rats just before fear testing reinstated FPS in these animals, whereas no such effect was observed in saline-treated animals (Borowski and Kokkinidis, 1998). This result, along with those mentioned earlier, add support for the notion that an activated mesoamygdaloid dopaminergic neural circuit is critically involved

in promoting excitatory CS-UCS learning and in facilitating memory retrieval processes that make the expression of conditioned fear possible.

In fact, direct manipulations of VTA DA neurons and receptors as well as D_1 and D_2 amygdaloid DA receptors has been shown to lead to significant disruptions in fear acquisition and/or expression in both the defensive freezing and FPS paradigms (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997; Waddington-Lamont and Kokkinidis, 1998; Nader and LeDoux, 1999b; Guarraci, et al., 1999; 2000; Greba and Kokkinidis, 2000; Gifkins, et al., 2002). With regards to the VTA, microinfusion of a 1.0 μg/side dose of the $D_{2/3}$ DA receptor agonist quinpirole hydrochloride directly into the A10 region of the VTA blocked the expression of FPS in rats conditioned to a visual CS (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997). Intra-VTA infusion of the $GABA_A$ receptor agonist muscimol (0.1μg/side) before final testing also blocked the expression of FPS in rats presumably through the GABAergic receptor mediated inhibition of VTA DA neurons (Munro and Kokkinidis, 1997; Westerink, Kwint, and de Vries, 1996; Westerink, Enrico, Feimann and de Vries, 1998). Along the same lines, infusions of quinpirole (e.g. 0.1 μg/side and 1.0 μg/side) into the VTA of rats prior to second order fear conditioning prevented rats from exhibiting defensive freezing behaviour to CS2 when fear testing was carried out the following day (Nader and LeDoux, 1999b). It has been hypothesised that quinpirole application to the VTA disrupts Pavlovian fear learning and expression by stimulating D_2 autoreceptors that reduce the activity of VTA DA neurons and by lowering the amount of DA that is available for D_1 and D_2 receptors in the amygdala (Nader and LeDoux, 1999b; White and Wang, 1984; Lacey, et al., 1987; Bernardini, et al., 1991; Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997).

Excitotoxic lesioning of the VTA blocks the expression of FPS in rats, while mild stimulation of the VTA has been shown to potentiate acoustic startle responses to white-noise bursts, an effect that also occurs after amygdaloidal stimulation (Borowski and Kokkinidis, 1996; Rosen and Davis, 1988a). Furthermore, exposure to environmental stressors (e.g. footshock and restraint), predator odors (e.g. TMT) or conditioned fear cues has been shown to trigger the production of the immediate early gene c-fos in the VTA, increase dopaminergic metabolism in the VTA, and raise the total amount of DA metabolites found in terminal regions such as the amygdala and prefrontal cortex (Beck and

Fibiger, 1995; Deutch, et al., 1985; Deutch, et al., 1991; Thierry, Tassin, Blanc, and Glowinski, 1976; Fadda, et al., 1978; Herman, et al., 1982; Ida, Tsuda, Seyoshi, Shirao and Tanaka, 1989; Coco, Kuhn, Ely, and Kilts, 1992; Deutch, Tam and Roth, 1985; Deutch, Lee, Gillham, Cameron, Goldstein, and Iadarola, 1991; Inglis and Moghaddam, 1999). This raises the possibility that reciprocal connectivity between the VTA and amygdala play a major role in mediating the stress-induced elevation in DA metabolites observed in the prefrontal cortex (Fadda, Argiolas, Melis, Tssari, Onali and Gessa, 1978; Herman, Guillonneau, Dantzer and Scatton, 1982; Claustre, Rivy, Dennis and Scatton, 1986; Goldstein, Rasmusson, Bunney and Roth, 1996; Deutch, Tam and Roth, 1985). For example, many of the fear or stress-induced elevations in DA metabolites in the prefrontal cortex can be attenuated by benzodiazepine treatment (Fadda, et al., 1978; Lavielle, Tassin, Thierry, Blanc, Herve, Berthelemy and Glowinski, 1978; Ida, et al., 1989; Coco, et al., 1992), destruction of amygdaloidal noradrenergic fibres innervating the VTA (Herve, Blanc, Glowinski, and Tassin, 1982), or lesioning of the central amygdaloid nucleus (Davis, Hitchcock, Bowers, Berridge, Melia, and Roth, 1994; Goldstein, et al., 1996).

Electrophysiological studies have generally strengthened the view that mesoamygdaloid DA systems are vital for fear learning and expression. For example, electrical stimulation of the central nucleus of the amygdala has been shown to alter the firing rates of VTA neurons (Maeda and Mogenson, 1981) and electrical stimulation of the VTA significantly increases startle responsiveness in rats (Borowski and Kokkinidis, 1996). Also, electrical stimulation of the VTA and SN DA neurons projecting to the BLA (Swanson, 1982; Loughlin and Fallon, 1983) caused fast firing neurons in the basolateral amygdaloid nucleus to increase their firing rates even more, whereas slow firing neurons in this amygdaloid region exhibited a marked decrease in activity (Rosenkranz and Grace, 1999). In a related manner, both VTA and amygdaloid kindling significantly increase expressions of fear in laboratory animals (Rosen, Hammerman, Sitcoske, Glowa and Schulkin, 1996; Stevens and Livermore, 1978) and VTA dopaminergic neurons firing patterns are altered during fear conditioning (Guarraci and Kapp, 1999). As mentioned above, intra-VTA infusion of either the $D_{2/3}$ receptor agonist quinpirole hydrochloride or the GABAergic agonist muscimol, two drugs that typically inhibit DA neurons and alter DA release (Westerink, Kwint, and deVries, 1996; White and Wang, 1984), have been shown to block

both the shock sensitisation of startle and FPS in laboratory rats (Gifkins, Greba and Kokkinidis, 2002; Munro and Kokkinidis, 1997; Borowski and Kokkinidis, 1996). Taken together, these findings seem to indicate that mesoamygdaloid neurodynamics may play a prominent role in fear learning and expression. In fact there seems to be a great deal of support for this claim as it has recently been shown that blockade of DA D_1 receptors in the amygdala impairs both the acquisition and expression of conditioned fear in both the defensive freezing and FPS paradigms (Guarraci and Kapp, 1999; Guarraci, Frohardt and Kapp, 1999; Waddington-Lamont and Kokkinidis, 1998; Greba and Kokkinidis 2000; Nader and LeDoux, 1999b).

In light of these facts, it is important to mention that considerable research evidence does point towards dopaminergic mediated events being involved in regulating amygdaloid functioning (Rosenkranz and Grace, 1999; Bissière, Humeau, and Lüthi, 2003). In fact all the critical elements and neurochemical characteristics that are needed for DA-mediated synaptic transmission appear to exist within the central, lateral, and basolateral nuclei of the amygdala (Boyson, et al., 1986; Dawson, et al., 1988; Wamsley, et al., 1989; Swanson, 1982; Loughlin and Fallon, 1983; Scibilia, et al., 1992; Fallon and Ciofi, 1992; Freedman and Cassell, 1994; Revay, Vaughan, Grant, and Kuhar, 1996; Asan, 1997; Asan, 1998; Swanson and Petrovich, 1998; Brinley-Reed and McDonald, 1999; Meador-Woodruff, et al., 1989; 1991; Fuxe, et al., 2003) and research indicates that DA metabolism is increased in the BLA during discrimination learning (Hori, Tanaka, and Nomura, 1993), exposure to predator odors (Morrow, et al., 2000) and in response to emotionally arousing environmental stressors (e.g. footshock and handling) or cues previously paired with aversive events (Herman, et al., 1982; Coco, et al., 1992; Inoue, Tsuchiya and Koyama, 1994; Inglis and Moghaddam, 1999; but see Morrow, et al., 2000 regarding amygdaloid DA metabolism in response to conditioned fear cues). Furthermore, recent electrophysiological studies have demonstrated that the firing rate of basolateral amygdaloid neurons can be influenced by DA agonist and antagonist drugs (Rosenkranz and Grace, 1999). More specifically, iontophoretic application of either DA or DA agonists (i.e. SKF 38393 and quinpirole) was shown to attenuate the firing rate of BLA neurons that had been activated by glutamate iontophoresis (Rosenkranz and Grace, 1999). Consistent with this result, co-microiontophoresis of DA and glutamate were reported to depress spontaneous spike

discharges that normally occur if glutamate is iontophoretically applied alone (Rosenkranz and Grace, 1999). It is noteworthy that the observed decrements in firing rate and spike activity induced by DA or DA receptor agonist iontophoresis could be blocked by systemic administration of haloperidol, a DA receptor antagonist and potent antipsychotic drug (Rosenkranz and Grace, 1999; Julien, et al., 1996). In addition, systemic administration of DA agonists (i.e. apomorphine, SKF 38393 and quinpirole) greatly augmented the firing rate and peak amplitude of "fast-firing" neurons in the BLA, whereas the firing rate of "slow-firing" basolateral amygdaloid neurons was completely suppressed by these pharmacological agents (Rosenkranz and Grace, 1999). The specific drug-induced alterations to firing rates wrought by apomorphine, SKF 38393, and quinpirole were reversed by the administration of dopaminergic antagonist drugs (raclopride, 0.25 mg/kg; haloperidol, 0.3 mg/kg; SCH 23390, 0.75 mg/kg) at systemic doses that have been shown to be effective in attenuating FPS and conditioned avoidance responding in rats (Rosenkranz and Grace, 1999; Davis, et al., 1993; Greba and Kokkinidis, 2000; Arnt, 1982; Sanger, 1987). For example, a 0.3 mg/kg dose of haloperidol was observed to reverse the enhancement in the firing rate of "fast-firing" BLA neurons caused by apomorphine (Rosenkranz and Grace, 1999). Similarly, the decrements in the firing rate of "slow-firing" basolateral amygdalar neurons induced by apomorphine were dramatically overturned by systemically administered raclopride (0.25 mg/kg) (Rosenkranz and Grace, 1999).

More recently, electrophysiological research has shown that DA regulates LTP induction in the lateral nucleus of the amygdala possibly by modulating or decreasing feedforward inhibitory signals originating from local interneurons (Bissière, et al., 2003). According to this scenario, reductions in inhibitory postsynaptic currents (IPSCs) arising from interneurons that target projection neurons may well result in less inhibitory tone in the amygdala and a substantial increase in excitatory signals being generated by output neurons that reside in the amygdala. For example, research data gathered by Bissière and associates (2003) demonstrates that application of DA to amygdaloid slice preparations decreases the amplitude of the feedforward inhibitory postsynaptic potentials (IPSPs) and IPSCs when voltage clamp and current clamp techniques were used to assess levels of inhibitory input on amygdala projection neurons. These researchers also found that monosynaptic IPSCs recorded from projection neurons were reduced by quinpirole and DA

application. This suggests that D_2 DA receptor-mediated events most likely influence inhibitory synaptic transmission within the amygdala either by decreasing inhibition onto projection neurons or by increasing inhibitory signals directed onto interneurons (Bissière, et al., 2003; Fuxe, et al., 2003). This later possibility is supported by the observation that feedforward inhibition onto lateral amygdaloid interneurons was augmented by DA and quinpirole application, which would have likely meant that any feedforward inhibition directed onto amygdala projection neurons would be greatly reduced (Bissière, et al., 2003). As a result, a greater amount of excitatory signals generated by amygdaloidal output neurons could be transmitted to the hypothalamus, nucleus accumbens, and various midbrain and brainstem regions that are involved in fabricating the many autonomic and behavioural responses that are symptomatic of a central fear state. It is not difficult to imagine how such a state of affairs could foster or enhance fear learning and expression during classical conditioning especially since DA metabolism increases in the amygdala and medial prefrontal cortex when animals are exposed to aversive sensory stimuli or cues that were previously associated with negative events (Herman, et al., 1982; Coco, et al., 1992; Inoue, et al., 1994; Inglis and Moghaddam, 1999; Morrow, et al., 1996; Morrow, et al., 2000). Perhaps the most important discovery made by Bissière, et al., (2003) was that the D_2 DA receptor antagonist sulpiride blocked DA's effect on IPSP amplitudes directed onto projection neurons. Sulpiride's ability to increase IPSPs in the presence of DA and ensure that the integrity of inhibitory output was maintained and transferred to projection neurons provides valuable insight into the way in which DA D_2 receptor antagonists block conditioned fear in laboratory animals and alleviate emotional disturbances in schizophrenic patients. At any rate, it appears as though amygdaloid dopaminergic receptors may be essential in Pavlovian fear learning and expression.

In fact, it has recently been shown that blocking DA receptors in the basolateral and central amygdala interferes with conditioned fear learning and expression. For example, Waddington-Lamont and Kokkinidis, (1998) demonstrated that the behavioural expression of FPS was completely abolished by bilateral infusion of the selective D_1 receptor antagonist SCH 23390 into the amygdala of rats just prior to final testing. However, infusion of SCH 23390 into the nucleus accumbens before final testing did not block the expression of FPS (Waddington-Lamont and Kokkinidis, 1998). In addition, both

peripheral administration (0.1 mg/kg, 0.5 mg/kg, and 1.0 mg/kg) and intra-amygdalar infusion (4.0 μg) of SCH 23390 prior to repeated fear conditioning and testing sessions blocked the acquisition and retention of FPS in rats (Greba and Kokkinidis, 2000). Saline-treated rats developed robust levels of FPS during the acquisition phase of the experiment as more training and testing session were delivered, whereas SCH 23390-treated rats did not (Greba and Kokkinidis, 2000). The learning deficit created by the antagonism of D_1 DA receptors with SCH 23390 during the acquisition phase also disrupted the retention of FPS when rats were subsequently tested for fear expression in a drug-free state forty-eight hours later (Greba and Kokkinidis, 2000). Thus it appears as though SCH 23390 interfered with the initial formation of CS-UCS fear associations during the training/testing phase and as such it prevented the consolidation of long-term fear memories from taking place. Moreover, the peripheral and intra-amygdalar application of SCH 23390 neither diminished reactivity to footshock nor did it prevent animals from exhibiting the shock-induced increases to acoustic startle (i.e. shock sensitisation of startle) that normally occur after a series of footshocks (Greba and Kokkinidis, 2000; Davis, 1989). This suggests that rats perceived the aversive nature of the shocks and expressed non-specific contextual fear and elevated arousal (i.e. shock sensitisation of startle) while being subjected to repeated fear-training and testing sessions during the acquisition phase of the experiment (Greba and Kokkinidis, 2000). In a very similar fashion, infusion of SCH 23390 (2.0 μg) into the amygdala before Pavlovian fear conditioning, prior to retention testing or both resulted in a significant attenuation of freezing behaviour to an auditory CS during retention testing (Guarraci, et al., 1999). More specifically, when high, medium, and low doses of SCH 23390 (2.0, 1.0, and 0.5 μg) were used to assess conditioned fear only the highest dose (e.g. 2.0 μg) was effective in blocking defensive freezing behaviour on the retention test. However, it is important to point out that low and medium doses did cause a slight dampening of conditioned defensive freezing but of course this was not statistically significant (Guarraci, et al., 1999). Also, intra-amygdaloid SCH 23390 infusions did not disrupt defensive freezing to contextual cues during acquisition training (Guarraci, et al., 1999) indicating that short-term fear and arousal generated by the footshock during tone + shock pairings was not impaired by this D_1 DA receptor antagonist.

An interesting discovery by Guarraci and colleagues (1999) was that intra-amygdaloid infusion of the D_1 DA receptor agonist SKF 82958 prior to fear acquisition training but not before retention testing led to an enhancement of freezing behaviour to an auditory CS. This finding is generally consistent with research that shows that DA agonists can enhance learning, memory consolidation, and performance in some behavioural tasks that rely on Pavlovian conditioning and motivational learning when they are administered before or shortly after training (Doty and Doty, 1966; Evangelista, and Izquierdo, 1971; Haycock, Van Buskirk, Ryan, and McGaugh, 1977; White and Viaud, 1991). For example, posttraining administration of amphetamine improves memory retention in avoidance discrimination and shuttle box avoidance tasks, whilst posttraining intraventricular infusions of DA enhances retention in passive avoidance tasks (Doty and Doty, 1966; Haycock, et al., 1977; Evangestilia and Iquierdo, 1971). Similarly, immediate posttraining infusions of either amphetamine or the D_2 receptor agonist LY171555 into the posteroventral region of the caudate nucleus (located just anterior to the AStr and the central and basolateral amygdaloid nuclei) improved the retention of a conditioned emotional response (i.e. suppression of drinking) to a visual CS (White and Viaud, 1991). Furthermore, amphetamine treatment has been reported to enhance the acquisition of FPS and active avoidance responding in laboratory rats (Bridger, and Mandel, 1967; Milner, 1974). Also, the enhancing effect on conditioned fear that Guarraci and associates (1999) obtained with intra-amygdala SKF 82958 infusions is compatible with the sensitising effect on FPS expression produced by DA agonists (Borowksi and Kokkinidis, 1994; Willick and Kokkinidis, 1995; Borowski and Kokkinidis, 1998) in that it suggests that DA receptor activation may strengthen CS-UCS fear associations and help facilitate memory retention.

In contrast, D_1 and D_2 DA receptor antagonists seem to have the opposite effect on fear learning, fear memory retrieval and fear retention as research has shown that peripheral or intra-amygdalar administration of these pharmacological agents impair avoidance responding, conditioned defensive freezing behaviour and FPS responding in rats (Arnt, 1982; Sherman, et al., 1982; Petty, et al., 1984; Davis, et al., 1993; Waddington-Lamont and Kokkinidis, 1998; Nader and LeDoux, 1999b; Guarraci, et al., 1999, 2000; Greba and Kokkinidis, 2000). Recently, it has been shown that intra-amygdaloid microinfusions of the D_2 DA receptor antagonist eticlopride blocks defensive freezing behaviour to an

auditory CS when administered prior to fear conditioning or before both fear conditioning and retention testing (Guarraci, et al., 2000). This raises the interesting possibility that in addition to D_1 DA amygdaloid receptors, D_2 receptors located within the amygdaloid complex could also be critically involved in the acquisition, expression, and reinstatement of FPS in rats. Since the functional role of amygdaloid D_1 and D_2 receptors in the acquisition, retention, and reinstatement of FPS has not yet been thoroughly investigated or clarified, this thesis will endeavour to accomplish this task by employing the FPS paradigm.

6.8: The FPS Paradigm and the Purpose of the Thesis

Physiological and psychological assessments based on changes in acoustic startle responding began in the 1930s. The experimental work in this field was initially used to study reflexive chaining, startle response latencies, and neural sensitisation and habituation of acoustic startle responses. This was undertaken in order to determine how auditory pathways and spinal reflexive responses are linked to other sensory systems (i.e. visual and somatosensory) and organized in the CNS (Lorente de Nó, 1933; Culler and Mettler, 1934; Prosser and Hunter, 1936; Landis and Hunt, 1939). For example, Prosser and Hunter (1936) discovered that footshock had the capacity to sensitise acoustic startle responding in rats, whilst Landis and Hunt (1939) demonstrated that startle responses could be triggered by visual, tactile, and auditory stimuli in humans and a variety of mammalian organisms. Perhaps the most influential work in the field of acoustic startle responding was conducted by Brown, Kalish and Farber (1951). These researchers discovered that an acoustic startle reflexive response normally elicited by exposure to a sudden loud noise burst could be greatly amplified in laboratory rats in the presence of a visual stimulus (e.g. light) that had been previously paired with a footshock (Brown, et al., 1951). The integration of Pavlovian fear conditioning techniques with acoustic startle responding has been very helpful in providing the neuroscience field with a scientifically valid and reliable behavioural assessment tool that has been quite successful in identifying the neural circuitry that is responsible for fear learning and expression (Hitchcock and Davis, 1986; 1987; 1991; Miserendino, et al., 1990; Sananes and Davis, 1992; Kim and Davis, 1993a,b; Davis, et al., 1993; Lee, et al., 1996a; Lee, Lopez, Meloni and Davis, 1996b; Campeau, et al., 1993;

Davis, 1992a,b,c; Fendt, et al., 1994 Walker and Davis, 1997; Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997; Waddington-Lamont and Kokkinidis, 1998; Shi and Davis, 1999; Walker and Davis, 2000; Greba and Kokkinidis, 2000; Lin, et al., 2001 Gifkins, et al., 2002; Fendt and Schmid, 2002).

The FPS paradigm or what is at times referred to as the potentiated-startle-effect has some distinct advantages over other behavioural paradigms that are commonly used to assess conditioned fear or fear-motivated learning (Davis and Astrachan, 1978; Davis, 1986). Firstly, FPS does not rely on instrumental learning, operant responding, or any behavioural tasks that contain elements of motor learning to demonstrate the presence of a central fear state, whereas most avoidance paradigms do (Davis, 1986; Davis, 1992a; also see Kim and Davis, 1993a,b; Lee, et al., 1996). This means that most avoidance paradigms have two or more types of learning occurring simultaneously while fear training is taking place. For example, Pavlovian conditioning may occur initially when a light or a buzzer signals the imminent arrival of a footshock, but once the rat learns this association and starts to perform avoidance responses, motivational, instrumental and motor learning occur as well and this can lead to difficulties in interpreting results especially when some experimental manipulations (e.g. systemic drug injection) can cause sensorimotor impairments. In contrast, FPS does not rely on such simultaneous forms of learning but rather on simple Pavlovian fear conditioning and testing techniques that are designed to look for changes in acoustic startle responding in the presence and absence of a sensory cue that had been repeatedly paired with an aversive event (i.e. footshock) at some earlier point in time (Davis, 1986; Davis, 1992a,b,c,) Thus, any alteration in FPS responding produced by systemic drug treatment, lesioning of a brain area or microinfusion of chemical compounds into a discrete brain region can be credited to changes in the internal emotional state of the organism and not to any disruption in voluntary motor learning (Davis, 1992a,b,c; Kim and Davis, 1993a,b; Lee, et al., 1996).

Second, FPS measures a rapid reflexive response in an almost all or nothing manner (i.e. much like the firing of a neuron) in order to determine the presence or absence of a central fear state (Davis, 1986; Davis, 1992a,b,c; Koch, 1999). This means that the FPS paradigm does not require animals to withhold a voluntary response or suppress on-going natural behaviours in order to determine whether fear is present or absent. In contrast, other

paradigms such as the defensive freezing paradigm and the conditioned lick suppression and social interaction tests view the suppression of responding as a behavioural indicator of fear or anxiety (Davis, 1986). This could be problematic in some instances, especially when pharmacological manipulations can by definition block fear learning and expression in some paradigms but actually appear to enhance fear in others. One such example that comes to mind are the results that show that metacoloprimide impairs fear-induced avoidance responding in rats (i.e. blocks fear) but can increase freezing behaviour as well (i.e. increasing fear) (Blackburn and Phillips, 1990; Blackburn, et al., 1992). Another potential problem is that certain pharmacological treatments may suppress behaviour, and therefore may appear to be anxiolytic but upon closer examination they may fail to block Pavlovian conditioned fear or reduce fear and anxiety in clinical populations (Davis, 1986).

Third, FPS paradigms incorporate a within-subjects design which basically means that startle amplitudes in the presence of a CS (i.e. light + noise trials) are compared to startle amplitudes in the absence of a CS (i.e. noise-alone trials) for each subject in an experiment (Davis, 1986). This process not only reduces between subjects variability but also makes it possible to calculate the level or magnitude of conditioned fear by simply subtracting the noise-alone trials from the light + noise for each animal (Davis, 1986). This capability to quantitatively calculate a magnitude of conditioned fear for each animal in an experiment is quite advantageous in the sense that it makes it possible to assign subjects in a fashion that is designed to create experimental groups that are approximately equivalent on levels of conditional fear prior to the implementation of a treatment.

Fourth, no footshocks are administered during actual testing for FPS when noise-alone and CS + noise trials are presented to subjects (Davis, 1986; 1992). This ensures that CS-induced elevations in startle amplitude cannot be attributed to shock presentation but rather to the association that developed as a consequence of light + footshock conditioning trials administered at an earlier time.

Fifth, the development of more advanced computerized training and testing equipment has given the FPS paradigm the ability to administer sequenced blocks of fear conditioning trials followed by short testing trials so as to examine how FPS develops over time (i.e. during an acquisition phase). This is quite advantageous in that it makes it possible to study the impact of experimental manipulations (i.e. drug infusions) on short-term versus

long-term fear memory (see Walker and Davis, 2000; Greba and Kokkinidis, 2000). Also, the ability to measure the shock sensitisation of startle during the acquisition phase of testing makes it possible to examine how drug manipulations affect shock arousal and/or short-term contextual fear. Moreover, the ability to measure shock reactivity makes it possible to determine whether or not a particular experimental manipulation (i.e. drug administration or lesion to a particular region) alters an animal's capacity to sense or detect pain. This is quite important since electrical shock is often used as a UCS or a fear-inciting catalyst in animal based psychological experiments (see Greba and Kokkinidis, 2000; Walker and Davis, 2000).

Sixth, FPS results obtained from laboratory animals are generalizeable to the human population since humans and a variety of animal species exhibit this reflexive response and by the fact that startle responding can be easily potentiated in humans and animals through classical conditioning techniques (Spence and Runquist, 1958; Ross, 1961; Davis, 1992a,b,c; Grillon, Ameli, Woods, Merikangas, and Davis, 1991; Lang, Bradley, and Cuthbert, 1990; Alfons, Hamm, Cuthbert, Globisch, and Vaitl, 1997; Davis, et al., 1989; Falls and Davis, 1994; Davis and Astrachan, 1978; Paschall and Davis, 2002). Furthermore, anxiolytic drugs that reduce fear and anxiety in humans generally block FPS in laboratory animals (Davis, 1979; Davis, 1986; Berg and Davis, 1984; Kehne, Cassella and Davis, 1988; Davis, 1992a,b,c; Davis, et al., 1993; Paschall and Davis, 2002), whereas anxiogenic agents (e.g. yohimbine and piperoxane) that increase anxiety in normal people and exaggerate it in anxious individuals actually augments FPS in rats (Davis, et al., 1993). Moreover, antipsychotic drugs that reduce paranoid ideations and symptoms associated with schizophrenia, block conditioned avoidance learning, and produce their effects by antagonising DA receptors, have also been shown to block FPS in rats when administered either systemically or directly into the amygdala (Davis, et al., 1993; Waddington-Lamont and Kokkinidis, 1998; Greba and Kokkinidis, 2000). Because FPS in rats so closely parallels what is observed in humans and is sensitive to pharmacological manipulation across different mammalian species, it can be stated that the FPS paradigm is a valid and reliable behavioural measure of fear and anxiety and is therefore an excellent technique for investigating the neural and biochemical systems that are involved in fear acquisition, expression and reinstatement.

Briefly, the FPS paradigm in conjunction with other Pavlovian fear conditioning paradigms has established that amygdaloidal NMDA, AMPA, GABA, benzodiazepine, and DA D_1 receptor subtypes are likely involved in fear acquisition, expression and memory consolidation processes (Miserendino, et al., 1990; Walker and Davis, 2000; Kim, et al., 1993; Walker and Davis, 1997; Lee and Kim 1998; Maren, et al., 1996b; Goosens and Maren, 2003; Maren, et al., 2001; Waddington-Lamont and Kokkinidis, 1998; Greba and Kokkinidis, 2000; Guarraci, et al., 1999; 2000; Wilensky, et al., 2000; Muller, et al., 1997; Helmstetter and Bellgowan, 1994; Helmstetter, 1993; Fanselow and Kim, 1994; Lee, et al., 2001). Nevertheless, the role of DA D_2 receptors in the acquisition and expression of FPS has not been thoroughly investigated even though initial reports have demonstrated that blockade of D_2 amygdaloid receptors with eticlopride disrupts defensive freezing behaviour (Guarraci, et al., 2000).

Another topic that has been left virtually unexplored concerns the neural mechanism and biochemical processes that contribute to fear reinstatement after extinction training has occurred. Although there have been several behavioural studies that demonstrated that exposure to a UCS or sensitising drugs can lead to a restoration of conditioned fear not much research has been directed at examining the underlying neural and biochemical processes that contribute to this behavioural phenomenon (Konorski, 1948; Rescorla and Heth, 1975; Westbrook, et al., 2002; Walker and Davis, 1997 in Walker and Davis, 2000; Borowski and Kokkinidis, 1998; Gewirtz, et al., 1997). This is quite unfortunate since fears, phobias, and PTSD all tend to return rapidly in humans if they are exposed to stressful situations, stimuli, or environments that are comparable to what they feared in the past (Foa and Dancu, 1994 in Zinbarg and Mineka, 2001; Fyer, 1998; Zinbarg and Mineka, 2001). From a clinical standpoint, a returning or reoccurring fear, phobia, or fear-induced stress disorder could be quite debilitating and costly to say the least. Therefore there is a real need to elucidate the neurophysiological and biochemical processes within the brain that may be contributing to fear reinstatement. Since it has been established that the amygdala is involved in Pavlovian fear learning and expression as well as in contributing to fear-extinction via NMDA-receptor-mediated processes, it is logical that this limbic region be chosen for scientific investigations designed to more fully examine the underlying neural

and biochemical factors that may be contributing to the reinstatement of FPS after extinction training.

The experimental work in this thesis is divided into three major components each of which has specific goals. The first component deals with the role of amygdaloidal DA D_1 and D_2 receptors and their contribution to the acquisition and retention of FPS in rats. Hence, the purpose of Experiments 1A and 1D is to investigate the role of amygdaloid DA receptors in Pavlovian fear learning and retention as measured by the FPS paradigm. Experiments 1B, 1C, and 1E have been included as short control experiments. Experiment 1B is designed to address any concerns about sensorimotor impairments that may arise due to intra-amygdaloid application of the D_2 receptor antagonist raclopride. Similarly, Experiment 1E was undertaken to determine if intra-amygdaloid administration of the DA D_1 receptor antagonist would impair normal levels of acoustic startle responding. Experiment 1C was included to provide proof that the visual stimulus (i.e. light) was in fact a neutral stimulus if it was not explicitly paired with an aversive footshock. It is therefore the goal of the first set of Experiments (1A to 1E) to clarify the role of amygdaloid D_1 and D_2 receptors in conditioned fear learning. It is hypothesised that blockade of amygdaloidal D_1 and D_2 DA receptors prior to Pavlovian fear conditioning will block the acquisition and retention of FPS in rats.

The second major component of this thesis deals with the neurobiochemical factors within the amygdala that may be involved in facilitating the reinstatement of FPS in fear-extinguished rats. As a result, this component is purposely designed to examine what contributions the amygdala is making to fear retrieval and reinstatement during unsignalled UCS administration. This component is subdivided into three parts. The first is strictly behavioural, whilst the second and third parts pharmacologically manipulate the amygdala directly. The aim of the behavioural study (i.e. Experiment 2) is to demonstrate that presenting fear-extinguished rats with five unsignalled footshocks (i.e. the UCS) in the fear conditioning and testing apparatus results in a robust reinstatement of FPS responding. This behavioural study not only seeks to replicate earlier reports that conditioned fear can be reinstated in fear extinguished animals if they are exposed to the UCS (i.e. footshock) but also helps to establish the set of training and testing protocols that will be used in later experiments that pharmacologically manipulate the amygdala (Pavlov, 1927; Konorski,

1948; Rescorla and Heth, 1975; Bouton and Bolles, 1979a,b; Westbrook, et al., 2002; Gewirtz, et al., 1997). It is predicted that fear-extinguished rats exposed to five unsignalled footshocks (i.e. the UCS) will exhibit a reinstatement of FPS, whereas non-shocked animals will not. Hence, it is felt that Experiment 2 will replicate the FPS reinstatement results obtained by Gewirtz and colleagues (1997).

Since D_1 and D_2 amygdaloid DA receptors have been shown to play an important role in fear learning and expression (Waddington-Lamont and Kokkinidis, 1998; Guarraci, et al., 1999; 2000; Greba and Kokkinidis, 2000) the goal of experiment 3A and 3B is to assess the role of these particular DA receptor subtypes in the reinstatement of FPS in rats. Similarly, NMDA and AMPA/kainate receptors have been shown to be essential for the acquisition and expression of classically conditioned fear (Miserendino, et al., 1990; Campeau, et al., 1992; Kim, et al., 1993; Walker and Davis, 1997; Gerwitz and Davis, 1997; Walker and Davis, 2000; 2002; Maren, et al., 1996; Lee and Kim, 1998; Fendt, 2001), LTP and excitatory synaptic transmission (Collingridge and Bliss, 1987; Reymann, et al., 1989; Bliss and Collingridge, 1993; Gean, et al., 1993; Shindou, et al., 1993; Phillips and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997; Mahanty and Sah, 1998; Wang et al., 2000; 2001; Maren, 1999; Schafe, et al., 2001; Kandel, 2000; Sah and Lopez de Armentia, 2003) and extinction learning (Falls, et al., 1992; Davis, 2000; Walker, Ressler, Lu, and Davis, 2002; Walker and Davis, 2002). It has been suggested that glutamatergic synaptic transmission taking place within the amygdala or in pathways that provide sensory input to the amygdala could be responsible for conditioned fear learning, fear memory consolidation, and fear expression (Maren, 1999; Lee and Kim, 1998; LeDoux, 2000; Fendt, 2001; Lee, et al., 2001; Phillips and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997). For this reason, the goal of Experiments 3C and 3D is to determine whether the blockade of NMDA and AMPA receptors in the basolateral amygdaloid complex will prevent the reinstatement of FPS in a population of fear-extinguished laboratory rats. With regard to dopaminergic systems, it is hypothesised that antagonism of D_2 and D_1 DA receptors in the amygdala will prevent the reinstatement of FPS. In a similar fashion, it is predicted that antagonism of NMDA and AMPA receptors located in the basolateral amygdaloid region will also block fear reinstatement as measured by the FPS paradigm.

Experiments 4 and 5 are specifically designed as control experiments for Experiments 3A to 3D and will be used to determine whether or not intra-amygdala infusions of dopaminergic and glutamatergic antagonist drugs twenty-four hours before final testing in non-fear extinguished rats will block FPS responding. It is speculated that pharmacological blockade of dopaminergic and glutamatergic amygdaloid receptors will have no deleterious effect on the expression of FPS exhibited by non-fear extinguished rats. The purpose of Experiment 6 is to control for the possibility of ataxia that may sometimes be produced by high doses of NMDA receptor antagonists (see Miserendino, et al., 1990 and Walker and Davis, 2000).

The third major experimental component of this thesis consists of a series of electrophysiological experiments specifically designed to elucidate the participation of the amygdala and other brain regions in facilitating conditioned fear reinstatement. The experiments making up this component are divided into two parts. The first part consists of a series of large studies that focus primarily on the amygdala, whereas the second part consists of a series of smaller studies that examine the role of individual brain regions that are intimately connected to the amygdala and that have been shown to be involved in conditioned fear expression. More specifically, Experiments 7A and 7B will ascertain if electrical stimulation of the amygdala in fear-extinguished rats will cause a reinstatement of FPS and alter after-discharge (AD) threshold levels in the amygdala.

It is well established that kindling of the amygdala induced by repeated electrical stimulation enhances emotionality and increases anxiety-like and fearful behaviours in laboratory animals. Furthermore, the most potent anxiogenic effects are obtained when stimulating electrodes used for kindling are placed in the basolateral and medial nuclei of the amygdala (Adamec and Morgan, 1994; Adamac, 1990; Helfer, Deransart, Marescaux, and Depaulis, 1996; Nieminen, Sirviö, Teittinen, Pitkänen, Airaksinen, and Riekkinen, 1992; Kalynchuk, Pinel, Treit, and Kippin, 1997; Kalynchuk, Pinel, and Treit, 1998; 1999; Kalynchuk, Pinel, Treit, Barnes, McEachern, and Kippen, 1998; Rosen, Hamerman, Sitcoske, Glowa, and Schulkin, 1996). For example, kindling of the amygdala typically increases defensive freezing behaviour during social interaction tests, reduces open-field activity and exploration, and exaggerates FPS responding in rats (Helfer, et al., 1996; Nieminen, et al., 1992; Rosen, et al., 1996; Wintink, Young, Davis, Gregus, and

Kalynchuk, 2003). In this connection it is noteworthy, that repeated seizure activity in epileptic patients set off by uninhibited neuronal discharge in the amygdala and nearby temporal lobe region can give rise to highly charged negative emotional states such as intense anxiety, panic, depression and of course overwhelming fear (Gloor, 1992; Depaulis, et al., 1997; Piazzini and Canger, 2001; Dodrill and Batzel, 1986). Since electrical stimulation of the central nucleus of the amygdala has been shown to elevate acoustic startle responsiveness (Rosen, 1988a; 1988b; 1990; Koch and Ebert, 1993) and increase many autonomic and behavioural responses that are indicative of a central fear state (Anand and Dua, 1956; Hilton and Zbrozyna, 1963; Kapp, et al., 1982; Applegate, et al., 1983; 1985; Gelsema, et al., 1987; Iwata, et al., 1987; LeDoux, 1987; Gloor, 1992), it is hypothesised that electrical stimulation of the amygdala will trigger a renewal of FPS responding in fear-extinguished rats and lower AD threshold levels in the amygdala. Conversely, it is expected that non-stimulated fear-extinguished rats will exhibit no fear reinstatement and will not display any significant reductions in AD threshold levels when EEG recordings are obtained from the amygdala.

The objective of Experiment 8 will be to determine whether or not electrical stimulation of the perirhinal/insular cortical region will lead to the reinstatement of FPS responding in fear-extinguished rats. Because the perirhinal/insular and temporal cortical areas are intimately connected with the amygdala, (McDonald, 1998; also see Chapter 3 of this thesis), have been shown to be involved in the expression of FPS (Rosen, Hitchcock, Miserendino, Falls, Campeau, and Davis, 1992; Campeau and Davis, 1995a,b; Falls, Bakken and Heldt, 1997), and provide polysensory input to the amygdala which includes auditory, visual, and nociceptive information (McDonald, 1998; Shi and Davis, 1998a,b; Shi and Cassell, 1999; Shi and Davis, 1999) it is postulated that electrical stimulation of the perirhinal/insular cortical region may instigate fear reinstatement in fear-extinguished rats.

Experiment 9 will seek to examine whether or not the reinstatement of FPS responding can be produced by electrical stimulation of either the dorsal PAG (dPAG) or the VTA. Experiment 10 will focus its experimental efforts on the caudal ventral PAG (vPAG). The rationale for studying the possible role of the VTA and PAG in fear reinstatement comes from the various scientific works that have verified the involvement of these two mid-brain areas in mediating fear expression (Borowski and Kokkinidis, 1996; Munro and

Kokkinidis, 1997; Gifkins, et al., 2002; LeDoux, et al., 1988; De Oca and Fanselow, 1995; Fanselow, et al., 1995; Fanselow, 1991; Fendt, Koch and Schnitzler, 1994; 1996; Fendt, 1998; Walker and Davis, 1997c; Walker, Cassella, Lee, Lima, and Davis, 1997). For example, there is a long standing research literature that has demonstrated that the PAG is involved in mediating conditioned defensive freezing behaviour (LeDoux, et al., 1988; De Oca and Fanselow, 1995; Fanselow, et al., 1995; Fanselow, 1991), defensive jumping, aggressive attack, flight behaviours (Bandler and Shipley, 1994; Behbehani, 1995; Bandler, 1982; Fernandez de Molina, et al., 1962) and FPS (Fendt, Koch and Schnitzler, 1994; 1996; Fendt, 1998; Walker and Davis, 1997d; Walker, Cassella, Lee, Lima, and Davis, 1997). In fact, it has been revealed that electrical or chemical stimulation of the PAG generally produces fear-like responses and flight behaviours, elevated noise-alone startle responses, and Pavlovian conditioned fear when used as an UCS (see Bandler, 1982; Borowski and Kokkinidis, 1996; Di Scala, Mana, Jacobs, and Phillips, 1987). Based on this research and the close anatomical association that the PAG has with the amygdala, it is logical to pursue investigations into how electrical stimulation of this mid-brain region might contribute to the reinstatement of FPS in rats.

With regards to the VTA, a plethora of research has demonstrated that this DA rich mid-brain region sends efferent projections to the amygdala (Swanson, 1982; Loughlin and Fallon, 1983; Brinley-Reed and McDonald, 1999; Oades and Halliday, 1987) and plays a critical role in fear expression (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997; Nader and LeDoux, 1999b; Gifkins, et al., 2002). For example, either neurotoxic lesioning of the VTA or functionally inhibiting VTA neurons with microinfusions of the GABAergic agonist muscimol has been shown to block the expression of FPS in rats (Borowski and Kokkinidis, 1996; Munro and Kokkinidis, 1997). More importantly, electrical stimulation of the VTA has been shown to augment acoustic startle responding in rats (Borowski and Kokkinidis, 1996) and alter the firing rates of fast and slow firing neurons in the amygdala (Rosenkranz and Grace, 1999). It is noteworthy that electrical stimulation of the VTA/SN region generally produced amygdaloid cell firing profiles that are similar to those generated by systemic administration of DA agonists (Rosenkranz and Grace, 1999) suggesting that DA turnover in the amygdala influences the activity levels of this limbic region. Specifically, electrical stimulation of the VTA/SN region caused "fast-

firing" amygdaloid neurons to increase their firing rates and "slow-firing" neurons to decrease their firing rates and these effects could be attenuated by systemic administration of the dopaminergic antagonist haloperidol (Rosenkranz and Grace, 1999). Consistent with these reports, electrically induced kindling of VTA neurons generates AD activity in the amygdaloid complex and triggers unconditioned fear responding in laboratory animals (Gelowitz and Kokkinidis, 1999; Stevens and Livermore, 1978). Based on the above discussions, it is reasonable to appraise the role of the VTA in the reinstatement of FPS. It is predicted that electrical stimulation of the VTA will cause a reinstatement of FPS responding in fear-extinguished rats when they are compared to the non-stimulated extinction control group.

To summarize, the purpose of the first experimental component of this thesis (i.e. Experiment 1A to 1D) is to investigate the involvement of amygdaloid D_1 and D_2 DA receptor subtypes in the acquisition and retention of FPS in rats. These experiments are mainly concerned with examining the contribution made by the mesoamygdaloid DA system to Pavlovian fear learning and CS-UCS fear memory formation. The goal of the second experimental component of this thesis (Experiment 2, Experiment 3A to 3D, and Experiment 4 to 6) is different in that its main thrust is to determine if dopaminergic receptors (D_1 and D_2) and glutamatergic receptors (NMDA and AMPA) located in the amygdala play a role in the synaptic processes that trigger the reinstatement of FPS in fear-extinguished rats exposed to unsignalled footshocks (i.e. UCS). The aim behind the third and final experimental component of this thesis is to determine if electrical stimulation of the amygdala and other brain regions intimately connected with the amygdala would result in a renewal of fear-induced startle responding in fear-extinguished rats.

It is hoped that the combined results of the pharmacological experiments in this thesis will enhance the scientific community's understanding of how D_1 and D_2 amygdaloid DA receptors facilitate the acquisition, expression and reinstatement of FPS. In addition to this, these experiments should provide further insight into how amygdaloidal AMPA and NMDA receptor-mediated synaptic transmission possibly revives fear memories inhibited by extinction training and contributes to the biochemical cascades that reinstate conditioned fear in mammals. The electrophysiological data gathered from the final experimental component of this thesis in conjunction with the experimental results

obtained from the other two components may even provide some additional clues that could be used to help resolve the question of whether or not the amygdala is the limbic brain region where fear memories are permanently stored. In doing so, this experimental work may start to provide some additional answers for questions such as; (i) Why do epileptic sufferers experience intense fearfulness and anxiety during seizures? (ii) How are changes in synaptic efficiency and/or synaptic transmission within certain amygdala-based neural circuits related to fear learning, fear-memory consolidation, and fear-memory retrieval and how do these events make it possible for an organism to make swift highly adaptive autonomic and behavioural responses to deal with sensory stimuli that signal danger? (iii) How are all these factors related to the recurrence of fear/phobias, the development and maintenance of posttraumatic stress disorder (PTSD) and much of the emotional and cognitive pathology associated with paranoid schizophrenia? In other words, how might the glutamatergic, dopaminergic, and GABAergic receptor-mediated processes and/or dysfunctions occurring in the amygdala and closely associated cortico-limbic areas of rats be linked to the emotional disturbances and recurring fear manifestations observed in those who suffer from schizophrenia, PTSD, and temporal lobe epilepsy?